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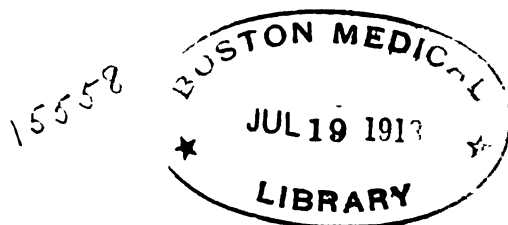
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A CONSIDERATION OF THE RELATIVE TOXICITY OF URANIUM NITRATE FOR ANIMALS OF DIFFERENT AGES. I.*

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PLATES 1 AND 2.

(Received for publication, January 31, 1917.)

The various ways in which the age of an organism expresses itself has received little consideration in the interpretation of many reactions that occur naturally and that are induced experimentally. The toxic effect of a substance experimentally introduced into an organism is usually interpreted either morphologically, by certain cell changes, or by some alteration in the functional capacity of an organ or a certain group of organs.

In a preliminary note¹ the observation was recorded that animals of different ages showed a variation in their response to the toxic effect of uranium nitrate when the poison was given subcutaneously in a constant quantity per kilo of body weight. The younger animals not only withstood the toxic effect of uranium for a longer period without developing an albuminuria and a glycosuria, but when these animals finally became both albuminuric and glycosuric, the quantitative output of these substances was much less in the younger than in the older animals.

In a more recent paper² the observation referred to has been confirmed in a second series of animals. A further observation has been made, that, judging by the time of appearance and the amount of acetone which occurred in the urine,

* Aided by a grant from The Rockefeller Institute for Medical Research.

¹ MacNider, W. deB., On the difference in the response of animals of different ages to a constant quantity of uranium nitrate, *Proc. Soc. Exp. Biol. and Med.*, 1913-14, xi, 159.

² MacNider, The inhibition of the toxicity of uranium nitrate by sodium carbonate, and the protection of the kidney acutely nephropathic from uranium from the toxic action of an anesthetic by sodium carbonate, *J. Exp. Med.*, 1916, xxiii, 171.

the older animals gave evidence of developing an acid intoxication much earlier than did the younger ones. In this paper it was also shown that a solution of sodium carbonate given intravenously would protect the kidney of an animal against the toxic effect of uranium, and that it was also possible to protect the kidney of a young animal acutely nephropathic from uranium against the toxic effect of an anesthetic by the use of a solution of sodium carbonate. The ability to furnish such a protection decreased as the age of the animal increased.

It is important to ascertain in a more detailed manner the way in which animals of different ages express their variation to the toxic effect of uranium. The present investigation is therefore primarily concerned with a study of acute uranium intoxications in animals of different ages as indicated by a disturbance in the metabolism of the animals, the severity of which shows a parallel with the age of the animal. During the course of such intoxications the animals become nephropathic. In these animals the kidney has been selected as an organ in which to study the functional and morphological variations which develop during the intoxication. The severity of these changes will be studied in relation to the age of the animal.

EXPERIMENTAL.

Dogs were employed in the experiments. The animals varied in age from pups of 8 months to dogs 8 years of age. The younger animals were raised in the laboratory kennels, while the older animals were obtained from people in the surrounding country who had raised the dogs and could vouch for their age within a few months.

The animals were placed in metabolism cages and given a liberal amount of bread with which was cooked a small amount of meat. The animals received 500 cc. of water daily by stomach tube. After a period of 3 days, which was allowed for normal observations, and during which time animals with a naturally acquired nephropathy or with glucose or acetone bodies in the urine could be excluded, the animals were given subcutaneously on 2 successive days 5 mg. of uranium nitrate per kilo of body weight. In all the animals the uranium intoxication was allowed to persist for 48 hours. At the end of this period the animals were either killed and autopsied, or the nephropathic animals were anesthetized and employed for cer-

tain functional studies which will be reported in Part II of this investigation.

The relative toxicity of uranium for these animals has been investigated by a study of the hydrogen ion content and alkali reserve of the blood, the tension of alveolar air carbon dioxide, and by the time of appearance and quantitative output of acetone and diacetic acid in the urine. The functional capacity of the kidney was ascertained by a study, at different periods during the intoxication, of the time of appearance and total output of phenolsulfonephthalein in the urine and by a study of the urea content of the blood.

The hydrogen ion determinations have been made by the indicator method recently devised by Levy, Rowntree, and Marriott.³ The alkali reserve of the blood and the determinations of alveolar air carbon dioxide have been made by the methods of Marriott.^{4, 5} The quantitative determinations of acetone and diacetic acid in the urine have been made by Folin's⁶ method as modified by Hart.⁷ The output of diacetic acid is expressed in terms of acetone. The blood urea determinations have been made by the method of Marshall,⁸ following the modification suggested by Van Slyke and Cullen.⁹ The phenolsulfonephthalein test for kidney function was conducted according to the method outlined by Rowntree and Geraghty.¹⁰

³ Levy, R. L., Rowntree, L. G., and Marriott, W. McK., A simplified method for determining variations in the hydrogen ion content of the blood, *Arch. Int. Med.*, 1915, xvi, 389.

⁴ Marriott, W. McK., A method for the determination of the alkali reserve of the blood plasma, *Arch. Int. Med.*, 1916, xvii, 840.

⁵ Marriott, The determination of alveolar carbon dioxide tension by a simple method, *J. Am. Med. Assn.*, 1916, lxvi, 1594.

⁶ Folin, O., On the separate determination of acetone and diacetic acid in diabetic urines, *J. Biol. Chem.*, 1907, iii, 177.

⁷ Hart, T. S., On the quantitative determination of acetone in the urine, *J. Biol. Chem.*, 1908, iv, 477.

⁸ Marshall, E. K., Jr., A rapid clinical method for the estimation of urea in urine, *J. Biol. Chem.*, 1913, xiv, 283.

⁹ Van Slyke, D. D., and Cullen, G. E., A permanent preparation of urease, and its use in the determination of urea, *J. Biol. Chem.*, 1914, xix, 211.

¹⁰ Rowntree, L. G., and Geraghty, J. T., An experimental and clinical study of the functional activity of the kidneys by means of phenolsulphonephthalein, *J. Pharm. and Exp. Therap.*, 1909-10, i, 579.

TABLE I.

Normal Animals.

No. of experiment.	Age.	Weight.	Water in 24 hrs.	Urine in 24 hrs.	Sulfonephthalein.		Blood urea.	P _a .	R. P _a .	Carbon dioxide tension.	Albumin, glucose, acetone, diacetic acid.
					Time of appearance.	Output in 2 hrs.					
	yrs.	kg.	cc.	cc.	min.	per cent	per cent			mm.	
1	8 mos.	15.9	500	769	5	68	0.012	7.45	8.0	40	0
2	"	10.5	500	509	8	71	0.012	7.4	8.1	40	0
3	1	15.81	500	660			0.015	7.45	8.1	37	0
4	1	19.0	500	960	5	67	0.015	7.4	8.1	43	0
5	2	14.68	500	630			0.015	7.45	8.05	43	0
6	3+	17.01	500	670	5	67	0.015	7.45	8.0	44	0
7	3+	9.8	500	762	4½	67	0.015	7.4	8.0	38	0
8	4+	13.15	500	670			0.015	7.45	8.0	39	0
9	5+	13.3	500	465	4	66	0.012	7.45	8.05	40	0
10	5+	18.2	500	520			0.020	7.45	8.0	40	0
11	8	10.6	500	640	5	73	0.016	7.45	8.0	40	0
12	8+	8.53	500	430			0.015	7.45	8.0	39	0

TABLE II.

Nephropathic Animals after 24 Hours.

No. of experiment.	Age.	Uranium nitrate per kilo.	Urine, 1st 24 hrs.	Sulfonephthalein.		Blood urea.	Acetone per 100 cc.	Diacetic acid per 100 cc.	P _a .	R. P _a .	Carbon dioxide tension
				Time of appearance.	Output in 2 hrs.						
	yrs.	mg.	cc.	min.	per cent	per cent	mg.	mg.			mm.
1	8 mos.	5	483	4	64	0.012	0	0	7.45	8.0	38
2	"	5	704	5	66	0.012	0	0	7.4	8.1	35
3	1	5	995			0.015	0	0	7.45	8.1	35
4	1	5	745	5	64	0.015	0	0	7.45	8.1	43
5	2	5	459			0.015	0	0	7.45	8.05	35
6	3+	5	1,150	7	54	0.016	0	0	7.35	8.0	40
7	3+	5	435	3½	51	0.015	0	0	7.4	8.0	35
8	4+	5	360			0.015	0	0	7.3	7.95	34
9	5+	5	1,060	8	31	0.015	0	0	7.4	7.95	36
10	5+	5	1,300			0.015	0	0	7.4	7.9	36
11	8	5	645	10	17	0.016	2.6109	3.4812	7.4	7.9	37
12	8+	5	520			0.015	2.6592	2.7559	7.25	7.9	34

TABLE III.

Nephropathic Animals after 48 Hours.

No. of experiment.	Age.	Uranium nitrate per kilo.	Urine, 2nd 24 hrs.	Sulfonephthalein.		Blood urea.	Acetone per 100 cc.	Diabetic acid per 100 cc.	P _H .	R. P _H .	Carbon dioxide tension.
				Time of appearance.	Output in 2 hrs.						
	Yrs.	mg.	cc.	min.	per cent	per cent	mg.	mg.			mm.
1	8 mos.	5	769	5	20	0.012	2.9493	2.2756	7.4	8.0	37
2	8 "	5	509	7	18	0.012	1.3054	4.0614	7.4	8.0	35
3	1	5	660			0.020	3.8680	2.3208	7.4	8.0	35
4	1	5	960	11	21	0.020	2.3691	4.3015	7.35	8.0	39
5	2	5	630			0.020	2.8043	4.1099	7.35	7.9	30
6	3+	5	670	10	24	0.020	1.4010	4.2064	7.35	7.9	35
7	3+	5	762	21	4	0.042	2.3045	4.1092	7.3	7.85	31
8	4+	5	595			0.026	3.7862	4.6416	7.3	7.85	32
9	5+	5	465	20	8	0.022	3.3845	9.6700	7.4	7.9	35
10	5+	5	520			0.022	0.3703	2.2241	7.3	7.85	34
11	8	5	640	20	4	0.030	4.9800	2.2241	7.25	7.85	34
12	8+	5	430			0.030	2.6592	7.3975	7.2	7.8	30

Tables I, II, and III give the normal findings in these animals of different ages for 1 normal day and for the 2 subsequent days of the uranium intoxication. The tables show the results obtained in twelve of the animals which have been selected according to their age as representative of the total number of animals employed in the experiments.

Observations on Normal Animals of Different Ages.

All the animals during the period of 3 days allowed for normal observations were freely diuretic. The total output of urine for the last day of observation varied in the respective animals from a minimum of 430 cc. to a maximum output of 960 cc. The urine was free from albumin, acetone bodies, and glucose, and did not contain casts.

Table I shows that in conducting the sulfonephthalein test for kidney function, the appearance of the dye in the urine varied slightly in the different animals. The earliest appearance was 4 minutes following the injection, while in one animal the appearance of the

dye was delayed for 8 minutes. The total output of the dye in a 2 hour period varied between a minimum of 66 per cent to a maximum output of 73 per cent. These variations have apparently no connection with the age of the animal. The highest output of the dye in this series of animals occurred in an animal 8 years old (Experiment 11, Table I).

The percentage of blood urea has been very constant for animals of all ages. In the normal dog the urea content per 100 cc. of blood has varied between 0.012 to 0.020 per cent.

The hydrogen ion determinations have been made from the oxalated whole blood. In such determinations the hydrogen ion concentration expresses both the volatile and non-volatile acid content of the blood. Recently Marriott⁴ has shown that frequently such readings are misleading, and that a more accurate conception of the changes in the hydrogen ion concentration of the blood may be obtained by removing the carbon dioxide from the dialysate, in this way obtaining a reading which represents any change in hydrogen ion concentration which may be due to non-volatile acids. Marriott refers to such a reading as the reserve alkali content of the blood ($R. P_H$).

In the normal animals of all ages the hydrogen ion concentration of the whole blood (P_H) has varied between 7.3 to 7.45, while the alkali reserve of the blood ($R. P_H$) has varied between 8.0 to 8.1.

The determinations of the tension of alveolar air carbon dioxide have shown a variation within the limits of normality, 37 to 44 mm. The Marriott method for such determinations was employed in seventy-one dogs and has given remarkably constant results.

From the observations which have been made on the normal animals we may conclude that the animals of different ages show no appreciable difference in their ability to eliminate sulfonephthalein, that the blood urea content in these animals is very constant, and that the animals even though varying much in age show naturally no tendency towards an acid intoxication.

Observations on Animals of Different Ages Intoxicated by Uranium Nitrate.

In the following study of the relative toxicity of uranium, it first became necessary to ascertain whether the weight of the animal, and therefore the total amount of the poison introduced, had any effect in determining the toxicity of the substance. By referring to Tables II and III it will be seen that this factor apparently does not influence the toxic response on the part of the animals. For instance the pup of Experiment 1, with a weight of 15.9 kilos, showed a delayed and slight toxic effect from uranium, while the animal of Experiment 12, 8 years old, and with a weight of only 8.53 kilos, showed clearly the toxic action of uranium during the first 24 hours following the initial injection, and by the end of the second 24 hour period was severely intoxicated. This type of observation has remained constant in all the animals employed in this study.

Following the first injection of uranium all the animals remained freely diuretic. In several of the animals (Experiments 6, 9, and 10) the output of urine was greatly increased.

A study of the sulfonephthalein output by the animals of different ages shows the following variations: There is only a slight change in the time of the appearance of the dye in the urine as compared with the normal animals. The delay in the time of appearance has been most marked in the older animals, while in the youngest animals the time of appearance has either been increased over the normal or remained unchanged.

In the animals of all ages the total elimination of sulfonephthalein in a 2 hour period is reduced. The reduction is slight in the young animals and marked in the old animals. In Experiment 1, a pup 8 months old, the total output of the dye was only reduced from the normal of 68 to 64 per cent. In Experiment 11, an animal 8 years old, a reduction occurred from the normal of 73 to 17 per cent.

Following the second injection of uranium there is a continuation of the relatively greater toxic effect of this poison for the kidneys of the older animals. The youngest animals which have been recorded in Tables II and III were 8 months old and from the same litter. These animals at the end of the 48 hour period of intoxication by

uranium had a sulfonephthalein output of 20 and 18 per cent respectively. The two oldest animals in which sulfonephthalein determinations were made show an output of the dye of 8 and 4 per cent.

The toxic effect of uranium for the kidney as shown by the reduction in the output of sulfonephthalein increases with the age of the animals.

The determinations of blood urea in the pathological as compared with the normal animals enable observations to be made concerning the degree of urea retention in the animals of different ages and also permit a study of the relation between the output of sulfonephthalein by the kidney and the amount of urea retained.

Following the first injection of uranium the percentage of blood urea remained practically constant in all the animals. There was no evidence of a retention of urea even though the output of sulfonephthalein had been greatly reduced. In Experiment 7, Table II, the sulfonephthalein output was reduced to 51 per cent while the percentage of blood urea remained constant. In Experiment 11, the sulfonephthalein output was reduced following the first injection of uranium from 73 to 17 per cent. The percentage of blood urea was uninfluenced by this degree of kidney injury and remained at the normal reading of 0.016 per cent.

Following the second injection of uranium a retention of blood urea was found to occur in all the animals over 8 months old. The animals which show the greatest reduction in the output of sulfonephthalein also show the highest retention of blood urea. The tables of experiments furthermore show that the decrease in the functional capacity of the kidney as indicated by a retention of blood urea increases with the age of the animal.

From these observations it would appear that as compared with the sulfonephthalein test for kidney function, the retention of blood urea is a much later manifestation of kidney inefficiency. When, however, the kidney shows serious impairment of function as indicated by the sulfonephthalein test, there occurs a retention of blood urea which shows a parallel with this test for renal function.

The degree to which the kidney may be impaired and yet show no evidence of a retention of blood urea is illustrated by the first two experiments of Table III. The output of sulfonephthalein by these

two young animals was 20 and 18 per cent, respectively, and yet the percentage of blood urea showed no variation from the normal.

In a previous paper² on uranium intoxications in animals of different age it was shown that the time of the appearance of acetone bodies in the urine and the relative amount of these bodies increased with the age of the animal. This observation was interpreted as indicating the development of an acid intoxication, and furthermore to furnish ground for the belief that such an intoxication was more readily induced by uranium in an old animal than in a young animal. This interpretation was strengthened by a series of experiments in which it was found possible to protect an animal against the toxic effect of uranium by the use of an alkaline solution intravenously. The degree of protection conferred by such injections was largely dependent upon the age of the animal. Young animals were more readily protected than were old animals.

Howland and Marriott¹¹ have recently called attention to the fact that the presence of acetone bodies in the urine is in itself insufficient evidence of a tissue acidosis. It is furthermore well known that in conditions of tissue acidosis the output of these bodies in the urine may be reduced as a result of a decrease in the functional capacity of the kidney. It has therefore seemed advisable in this series of animals to ascertain whether there was any evidence of an acid intoxication other than that shown by the appearance of acetone bodies in the urine. With this object in view determinations of the hydrogen ion content of the blood, the alkali reserve of the blood, and the carbon dioxide tension of alveolar air have been made to determine first, if by these methods any evidence can be obtained of a tissue acidosis, second, if the degree of acid intoxication shows any parallel with the quantitative output of acetone bodies in the urine, and, finally, if the degree of intoxication as indicated by these different methods shows any variation with the age of the animal.

Reference to Table II shows that following the first injection of uranium none of the animals under 8 years of age had a urine which contained acetone or diacetic acid. However, the two oldest ani-

¹¹ Howland, J., and Marriott, W. McK., A discussion of acidosis. With special reference to that occurring in diseases of children, *Bull. Johns Hopkins Hosp.*, 1916, xxvii, 63.

imals of the series, Experiments 11 and 12, one 8 years old and the other 8 years and a few months old, showed both acetone and diacetic acid in the urine at this early period of the uranium intoxication. By referring to Table II it will also be observed that the appearance of acetone and diacetic acid in the urine of the two oldest animals coincides with the development of other indications of an acid intoxication. These animals also show an increase in the hydrogen ion concentration of the blood, a reduction in the alkali reserve of the blood, and a decrease in the tension of alveolar air carbon dioxide.

A study of the animals of Experiments 8, 9, and 10, which have varied in ages between $4\frac{1}{2}$ and 5 years and 2 months, shows that an acid intoxication may exist without the appearance of acetone bodies in the urine. All these animals had a urine which was free from both acetone and diacetic acid and yet all three of the animals gave other evidence of a beginning acid intoxication. It would therefore seem that the appearance of acetone and diacetic acid in the urine may indicate a beginning acid intoxication from uranium. On the other hand, the absence of these bodies from the urine does not exclude a tissue acidosis, for when other tests are employed such a state may be found to exist.

During the second 24 hours of the uranium intoxication the animals of all ages showed the presence of acetone and diacetic acid in the urine. In so far as the development of an acid intoxication can be determined by the presence of these substances in the urine, at this stage of the intoxication the tendency of the animals to develop an acidosis has extended so as to include not only the old animals but the animals of all ages.

The quantitative output of acetone and diacetic acid shows no constant increase with the increasing age of the animal. In general the combined acetone and diacetic acid output is greater in an old animal than in a young animal, but throughout the series of experiments numerous instances have been observed in which there is no true correlation between the age of the animal and the acetone and diacetic acid content of the urine. For example, in Experiment 2, Table III, in a pup 8 months old, the urine, following the second injection of uranium, contained 1.3054 mg. of acetone and 4.0614

mg. of diacetic acid in terms of acetone per 100 cc. of urine. In Experiment 10, in an animal 5 years and 2 months old, the output of acetone was only 0.3703 mg. per 100 cc. of urine, and the output of diacetic acid which was 2.2241 mg. per 100 cc. was but slightly over half the quantity found in the urine of the young animal.

A study of the relation between the quantitative output of acetone bodies in the urine and the changes in blood and alveolar air shows that when these bodies appear in the urine changes also occur in the blood and alveolar air indicative of a beginning acid intoxication.

The experiments also show that at this stage of the intoxication there is no correlation between the total output of acetone bodies and the other indications of the development of a tissue acidosis. For instance, in the animal of Experiment 10, at the end of the uranium intoxication the hydrogen ion content of the blood had been increased to 7.3, the alkali reserve of the blood was reduced to 7.85, and the tension of carbon dioxide in alveolar air gave a reading of 34 mm. The urine, however, showed a remarkably low output of both acetone and diacetic acid, acetone 0.3703 mg. and diacetic acid 2.2241 mg. per 100 cc. of urine.

From this review of the output of acetone and diacetic acid by animals of different ages intoxicated by uranium, the following conclusions may be drawn. The oldest animals show the toxic effect from uranium by the appearance of these bodies in the urine 24 hours before they appear in the urine of the younger animals. When animals of any age show the presence of these substances in the urine, they also show other evidence of a beginning acid intoxication.

The acid intoxication which develops from uranium cannot be solely ascribed to the formation of acetone bodies. These substances may fail to appear in the urine when there is other evidence of a beginning acid intoxication, and when as shown by the sulfonephthalein test the kidney has not become functionally inactive to such a degree as to cause a retention of these bodies. We must therefore conclude that the acid intoxication which develops from uranium is certainly dependent in part upon the formation or retention of acids other than those of the acetone series. Finally, it has been shown that the quantitative output of acetone and diacetic acid bears no constant relation to the age of the animal, and also that there is no

definite correlation between the quantitative output of acetone bodies and the degree of acid intoxication which may be demonstrated by other tests which have been employed for this purpose.

The variation in the toxicity of uranium for animals of different ages is more clearly expressed in changes in the acid-base equilibrium of the blood than by any other response which has been induced during the course of the intoxication.

Reference to Table II shows that following the first injection of uranium the animals under 4 years of age maintain a hydrogen ion content of the blood which varies only slightly from the normal. None of these animals have shown any change in the alkali reserve of the blood. In contrast with this indication of stability on the part of the animals under 4 years of age, all the animals over 4 years old have not only shown an increase in the hydrogen ion content of the blood but the alkali reserve of the blood has constantly shown a depletion. In these older animals in which the alkali reserve has been reduced, the tension of carbon dioxide in alveolar air has varied between 37 to 34 mm. In the younger animals in which the alkali reserve has remained unaffected the tension of carbon dioxide has varied between 43 and 35 mm.

The relative degree of acid intoxication induced in the animals of different ages is even more clearly shown following the second injection of uranium. By this stage of the intoxication, all the animals over 1 year of age not only show an increase in the hydrogen ion content of the blood, but the alkali reserve of the blood has been severely drawn upon. The younger animals, those under 1 year of age, have either shown no change in the hydrogen ion content of the blood, or in the few experiments in which these readings have varied, the hydrogen ion content has not been increased above 7.4, a point which may be considered within the limit of normal variations. In these younger animals the alkali reserve of the blood has either remained unaffected, or it has not been reduced below 8.0.

A study of Tables II and III not only indicates the differences in the hydrogen ion content and alkali reserve of the blood, which, as has been pointed out, exist between the animals of two age limits, those under 1 year of age and those over 1 year of age, but it also serves to demonstrate that as the animal increases in age there is a pro-

gressive increase in the degree of acidosis. For instance, at the end of the 2nd day of the uranium intoxication the animal of Experiment 6, 3 years and 2 months old, had a hydrogen ion concentration of 7.35 and an alkali reserve of 7.9, while the animal of Experiment 12, 8 years old, had a hydrogen ion content of 7.2 and an alkali reserve of 7.8.

The determinations of carbon dioxide tension in alveolar air of the animals of different ages at the end of the 2nd day of the uranium intoxication show that the older animals that have developed a reduction in the alkali reserve of the blood have an alveolar air carbon dioxide tension which varies between 35 to 30 mm. The younger animals in which no change has taken place in the alkali reserve, or in which the alkali reserve has not been reduced below 8.0, show a tension of carbon dioxide which varies between 39 to 35 mm.

From the observations the conclusion appears clear that one of the constant manifestations of the toxic effect of uranium is the development of an acid intoxication, and that the severity of this intoxication is associated with the age of the animal. The older animals develop a severer intoxication than do the younger animals.

The Pathology of the Kidney in Animals of Different Ages Intoxicated by Uranium.

At the end of the 2nd day of the uranium intoxication, twelve of the animals which have been employed in the experiments were killed and kidney tissue was at once fixed in 10 per cent formalin, Zenker's fluid, and in mercuric chloride-acetic acid to be used in the histological study. The animals from which tissue was obtained varied in age between 8 months, and 7 years and 4 months.

In the foregoing discussion of the relative toxicity of uranium in animals of different ages it has been shown that the toxicity of uranium increases with the age of the animal, and furthermore that the degree of toxicity of this substance is associated with the severity of the acid intoxication which develops in these animals. The following histological study has been undertaken with the object of ascertaining whether there is any pathological difference in the kidneys of animals which have shown very mild grades as contrasted

with severe grades of acid intoxication. The functional tests that have been employed have shown a correlation between the degree of acid intoxication and the ability of the kidney to eliminate sulfonephthalein and urea.

In the histological study it has not been found possible to differentiate between kidneys which were obtained from animals near the same age. In kidneys which were obtained from animals that varied 3 years or more in age there has been found such a difference in the pathological response of the organs as to permit a classification of the kidneys into two groups. The first group is represented by kidneys of animals not over 1 year of age and the second group by kidneys of animals that were over 3 years of age.

The kidneys of the younger animals show no evidence of damage to the vascular tissue. The glomerular capillaries are usually well filled with blood. No exudate has been observed in the subcapsular space or between the tubules. The connective tissue of the kidney has not been edematous. The endothelial nuclei of the glomerular vessels and of the cells lining the capsule of the glomerulus stain normally and show no evidence of degeneration or of proliferation.

The epithelium of the tubules, and especially of the convoluted tubules, shows a definite shrinkage. The lumen of the tubules is prominent and usually free from albuminous material. The nuclei of these cells are large in proportion to the surrounding cytoplasm and stain intensely (Fig. 1).

No stainable fat has been observed in the convoluted tubules. In frozen sections of the kidney stained for fat by Herxheimer's modification of the Scharlach R stain, there has been found in the ascending and descending limb of Henle's loop a very small amount of fat which appears as dust-like particles.

The pathology of the kidney of the older group of animals shows a similarity with the younger group in that no demonstrable changes have taken place in the vascular element of the kidney. The epithelium of the tubules shows the following changes which serve to separate the kidneys of the animals of different ages into the two groups.

The cells of the convoluted tubules show an increase in volume which is variable. In some of the animals the swelling has been marked, while in other animals this change has taken place to a

much less extent. Associated with the swelling of the cells the cytoplasm becomes distinctly granular and occasionally shows vacuolation. The free border of the cells appears ragged. The nuclei are decreased in size and stain less intensely (Fig. 2).

In such cells, unless the animal is very old, no stainable fat has been demonstrated. However, in the loops of Henle the amount of stainable fat has greatly increased over that which has been observed in the younger group of animals. The fat appears as large droplets which frequently coalesce. The difference in the amount of stainable fat in the kidneys of these two groups of animals of different ages is the most constant and striking histological variation.

When these differences in the pathology of the kidney are compared with the variations in the degree of acid intoxication and the alterations in the functional capacity of the kidney that are shown by the two groups of animals, the following differences are found to exist.

The kidneys of the younger group of animals in which the epithelium is histologically well preserved except for the appearance of a slight amount of fat in the tubules of Henle show only slight evidence of a beginning tissue acidosis and the functional capacity of the kidney is much less impaired than is the case with the second group of older animals.

The kidneys of the older animals which give histological evidence of a beginning degeneration of the convoluted tubules and which have shown a marked accumulation of fat in the tubules of Henle, show a much severer grade of acid intoxication than the younger animals, and also show that the functional capacity of the kidney has been severely impaired.

In the older group of animals there is an association between the degree of epithelial injury and the amount of fat in the kidney, with the severity of the acid intoxication, and the extent to which the functional capacity of the kidney has been affected.

As a result of this observation the question naturally arises: Does the kidney injury develop primarily, and the tissue acidosis result from a retention of acid bodies which the kidney is unable to remove, or is the kidney injury secondary to, and dependent upon the acid intoxication, resulting from the administration of uranium?

This question has been in a measure answered in the communication² previously referred to. If young animals that are being intoxicated by uranium are given a solution of sodium carbonate intravenously they fail to develop an acid intoxication and the renal epithelium either shows no evidence of degeneration, or the degenerative changes are slight when compared with the epithelial damage which occurs in animals unprotected by the use of an alkali.

CONCLUSIONS.

1. The toxic effect of uranium when given in a constant quantity per kilo of body weight is variable. This variation has been constantly associated with differences in the age of the animals. Uranium is more toxic for an old animal than for a young animal. The establishment of this fact, namely, that the age of an animal may modify the toxicity of a substance, should be taken into account in establishing by animal experiment the degree of activity of substances which are to be used for therapeutic purposes.

2. The toxic effect of uranium nitrate is constantly associated with its ability to induce a tissue acidosis. A severer grade of acidosis is induced in an old animal from uranium than is induced in a young animal.

It would appear that in the response of dogs of different ages to uranium the animals represent a reaction system to this substance which shows an increasing susceptibility as the animal advances from youth to senility.

Insufficient experimental data are as yet available to allow a discussion of the mechanism by which such an acid intoxication is produced.

3. The toxic effect of uranium is manifested locally by certain degenerative changes in the kidney. These changes are more marked in the kidney of an old animal than they are in the kidney of a young animal.

Associated with the severer kidney changes which are especially characterized by a beginning swelling of the renal epithelium and by an accumulation of stainable fat in these cells is the development of a severe grade of tissue acidosis.

4. The functional capacity of the kidney shows a parallel with the degree of acid intoxication and with the severity of the histological changes which have developed in the renal epithelium.

EXPLANATION OF PLATES.

PLATE 1.

FIG. 1. Camera lucida drawing, Leitz oc. 1, obj. 6. The figure is from the kidney of the young dog of Experiment 3. The glomerular vessels are well filled with blood. The epithelium of the convoluted tubules, *a*, shows a distinct shrinkage; the nuclei are large and stain intensely. The lumen of the tubules is prominent. At *b* are shown tubules in which the epithelium is beginning to show an early swelling. The animal gave little evidence of an acid intoxication.

PLATE 2.

FIG. 2. Camera lucida drawing, Leitz oc. 1, obj. 6. The figure is from the old animal of Experiment 11. The glomerulus shows no degenerative changes. The epithelium of the convoluted tubules, *a*, appears granular, stains less intensely than the epithelium of the younger animal of Fig. 1, and the nuclei are small and hypochromatic. The free borders of the cells are ragged. At *b* are shown tubules in which the cells are swollen. At *c* the epithelium shows vacuolation. The animal had developed a severe acid intoxication.

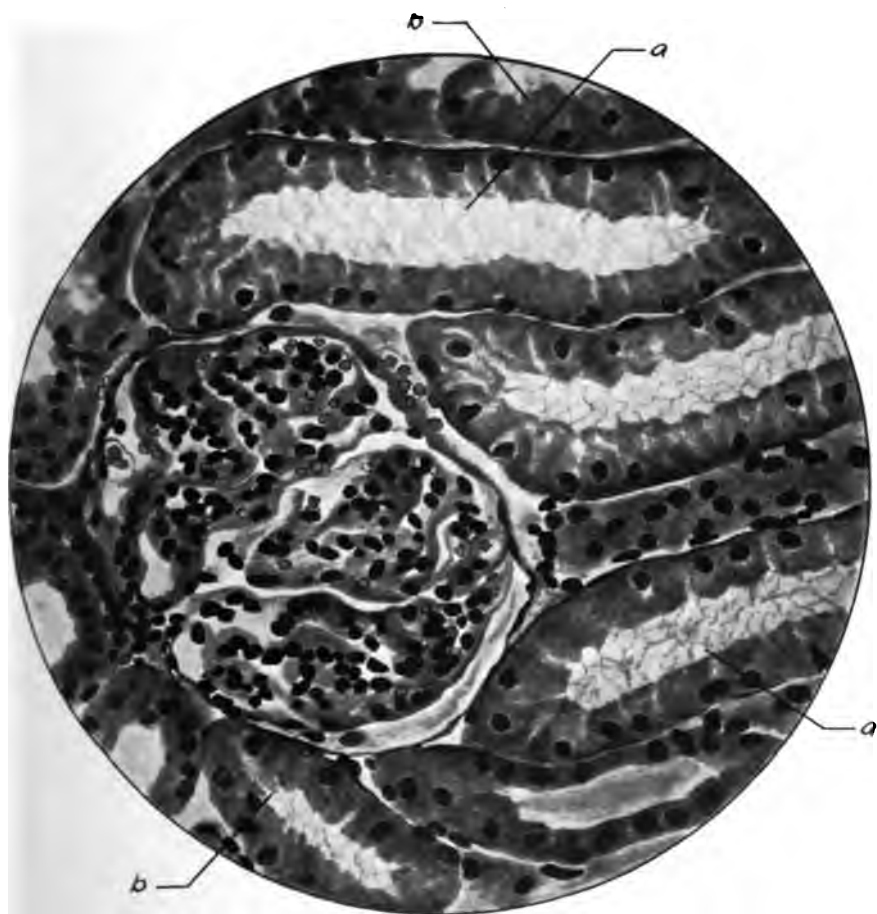


FIG. 2.

(MacNider: Toxicity of uranium. II.)

THE RELATION OF MOSQUITOES AND FLIES TO THE EPIDEMIOLOGY OF ACUTE POLIOMYELITIS.

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Of several hypotheses concerning the mode of conveyance of poliomyelitis, that of direct contact, supported by Flexner, appears to be the only one which stands on a substantial experimental basis.^{1,2} Although in all epidemics instances have been reported in which more than two members of a family were victims of the disease, as a rule only one out of several children in a family is attacked. This peculiar feature of the epidemiology of poliomyelitis seemed to support the theory that the disease may be inoculated by the bite of an infected insect rather than transmitted directly from one individual to another.

Typical examples of insect transmission of disease are found in yellow fever, malaria, spirochetoses, trypanosomiasis, and other tropical fevers. Rosenau and Brues³ in 1912 announced their success in transmitting poliomyelitis from an infected to a normal *Macacus* monkey by means of the bites of stable-flies (*Stomoxys calcitrans*). Their experiment consisted in allowing many thousands of the flies to feed first on an infected monkey and immediately afterwards on a normal monkey. The transmission was mechanical, but not in the sense of an intermediary host. Anderson and Frost⁴ confirmed Rosenau's finding in a

¹ Flexner, S., The mode of infection and etiology of epidemic poliomyelitis, *Am. J. Dis. Child.*, 1915, ix, 353.

² Flexner, The nature, manner of conveyance and means of prevention of infantile paralysis, *J. Am. Med. Assn.*, 1916, lxvii, 279.

³ Rosenau, M. J., and Brues, C. T., Some experimental observations upon monkeys concerning the transmission of poliomyelitis through the agency of *Stomoxys calcitrans*, *Tr. XVth Internat. Cong. Hyg. and Demography*, Washington, 1913, i, 616.

⁴ Anderson, J. F., and Frost, W. H., Transmission of poliomyelitis by means of the stable fly (*Stomoxys calcitrans*), *Pub. Health Rep., U. S. Mar. Hosp. Serv.*, 1912, xxvii, 332.

limited number of instances, and then later failed to obtain further positive results. Howard and Clark⁵ were unable to transmit the infection from an infected to a normal monkey by the bites of *Stomoxys calcitrans*. The peculiarity of conveyance of the infection is far from being solved by the theory of transmission by these blood-sucking flies.

The most plausible hypothesis so far advanced seems to be that offered by Amoss and Taylor,⁶ who, having experimentally demonstrated the existence of a protective principle in the secretion of the nasal mucous membranes of normal individuals, consider that the incidence of infection may to a considerable extent depend upon the condition of these mucous membranes; that is, as long as they remain intact, the virus will be destroyed before it can become established in the individual. The disturbance of this natural protective mechanism exposes to infection, but not the presence or absence of the virus alone. They were able to show that the protective substance in question was more generally and abundantly present in adults than in young children.

The sudden disappearance of poliomyelitic cases with the return of cold weather is often used as an argument in favor of insect transmission of the disease. Flies and mosquitoes have constantly been under suspicion. While the elimination of these insects from our households is desirable as a matter of routine cleanliness, yet without a systematic experimental investigation they should not be accepted as a factor in the epidemiology of poliomyelitis. Indeed, it would be unwise if, through a misapprehension of the facts, the attention of health officers and laity alike were to be diverted from the real source of danger.

Scope and Mode of Experiments.

The question whether a given variety of insect plays a part in the spread of poliomyelitis is within reach of exact determination, since these insects are amenable to artificial propagation through many generations. During the past several months, we have been able to collect and propagate in tanks in the laboratories a quantity of *Culex pipiens* from sewer waters near Jersey City⁷ and in

⁵ Howard, C. W., and Clark, P. F., Experiments on insect transmission of the virus of poliomyelitis, *J. Exp. Med.*, 1912, xvi, 850.

⁶ Amoss, H. L., and Taylor, E., Neutralization of the virus of poliomyelitis by nasal washings, *J. Exp. Med.*, 1917, xxv, 507.

⁷ We are greatly indebted to Dr. Headlee, chief entomologist of the New Jersey Agricultural Experiment Station, at New Brunswick, and to Dr. Chidester

Queens County, Long Island. It was our intention to study several well known genera of mosquitoes at the same time, but owing to the lateness of the season, none of the *Aedes* or *Anopheles* species were available. The only variety which we were able to obtain was *Culex pipiens*. Experiments were also performed to determine whether the common house-fly or the bluebottle fly is able to take up the poliomyelitic virus and make it increase within its body.

There are at least two totally different ways of attacking the problem. The first method is to feed young growing larvæ with the active poliomyelitic virus by putting it in the polluted water in which they are being grown. When the adult mosquitoes have hatched out, they may be allowed to feed on a normal *Macacus* monkey. The second procedure is that ordinarily practised by investigators and consists in allowing the mosquitoes to feed first on an infected animal and then, within a certain length of time, on a normal animal.

The experiments with non-biting flies had to be carried out by still another method. They were allowed to feed on the nervous tissues containing an enormous amount of the poliomyelitic virus until they pupated. Part of the pupæ were allowed to hatch. Both the pupæ and the imagoes were then crushed together and the emulsion was filtered through a Berkefeld filter. The clear filtrate was introduced intracerebrally and subsequent events were observed.

Experiments with Mosquitoes Hatched in Polluted Water Experimentally Contaminated with the Virus of Poliomyelitis.

To twelve jars, covered with a wire net cage and containing mosquito larvæ of various ages in ordinary stagnant water, were added varying amounts of the brain or cord emulsions or their filtrates derived from two monkeys that died of experimental poliomyelitis. The virus was introduced every 24 hours for a period of 10 days. The temperature of the laboratory was kept at about 70°F. and the

of Rutgers College, New Brunswick, for their assistance and instruction as to the propagation of mosquitoes in the laboratory. We wish to express our gratitude also to Mr. Winchell of Rutgers College, who assisted us in collecting the specimens in the field.

relative humidity at about 40 per cent. Numbers of adult mosquitoes, both male and female, hatched out from the jars every day. They were carefully collected and used for transmission experiments on normal monkeys. The rate of hatching from day to day is shown in Table I.

TABLE I.

Date.	Female.	Male.
<i>1916</i>		
Oct. 31	21	20
Nov. 1	12	9
" 2	11	2
" 3	17	8
" 5	10	12
" 6	15	8
" 7	30	18
" 8	25	9
" 9	13	18
Total.....	154	104

The first transmission experiment was performed with mosquitoes on two young *Macacus rhesus* monkeys. As the mosquitoes attack the monkeys only during the night, the experiment was begun at dusk and discontinued the next morning. The results were as follows: Of 150 females and 60 males put into the cage, 114 females were fully engorged with the blood, 23 females were apparently not engorged, and 52 males were still living. Some of the mosquitoes had been killed by the monkeys.

Close observation was maintained on the monkeys for 21 days, but no sign of poliomyelitis developed nor has any been noticed up to the present time.

Some of the engorged females sooner or later laid eggs and perished, while some died without oviposition. A number of female mosquitoes hatched out of the egg boats and also from the virus-containing water were kept alive for about 3 weeks and then allowed to bite two young *Macacus* monkeys. There were about 40 well engorged specimens when examined the following morning. The monkeys showed no symptoms of poliomyelitis.

These experiments demonstrate that the mosquitoes raised in water containing the virus of poliomyelitis in large quantities cannot transmit the infection by their bites to normal *Macacus* monkeys. There is no indication that the virus enters and multiplies in the body of mosquitoes, even if 3 weeks are allowed to elapse between the time of hatching and that of the biting experiment. The offspring of these females are not capable of transmitting the disease to the monkey.

Experiments to Determine Whether the Females Well Engorged with the Blood of Infected Monkeys Will Ever Be Capable of Transmitting the Infection by Their Bites to Normal Macacus Monkeys.

In order to decide this point, eight groups of mosquitoes, some obtained from the vicinity of Jersey City, and some from Queens County, Long Island, were allowed to feed on several *Macacus* monkeys inoculated for this purpose. To insure ourselves of including different stages of the infection, feedings were made every 24 hours after the inoculation of the virus into the monkeys and were extended into the paralytic stage, which of course varied in different animals from 6 to 9 days, according to the activity of the virus and the quantity injected. The protocols of these feeding experiments show the time which elapsed between the feedings and the transmission experiments. The term "feeding mosquitoes" is used merely to denote that these mosquitoes have sucked blood from an infected monkey.

Four out of the eight groups were tested on November 20; that is, 1 to 2 weeks after the time of feeding. The remaining four groups were allowed to bite after having been kept from 2 to 3 weeks. It must be mentioned that the engorged females do not survive for any length of time after oviposition, and comparatively few specimens remained alive in the second series of groups.

For the purpose of transmission two young *Macacus* monkeys were employed for each of the two experiments (Groups 1 to 4 and 5 to 8). We also inoculated 2 cc. of sterile normal horse serum into the intrathecal space in order to derange the protective mechanism which the meninges provide against the penetration of the virus

into the central nervous system in experimental poliomyelitis in monkeys. It may be recalled that Flexner and Amoss⁸ demonstrated that a fraction of the virus, intravenously introduced, will readily localize in the central nervous system when the meninges are previously disturbed by the inoculation of various substances, although the monkey may bear a multiple of such doses with impunity if there has been no previous meningeal injury.

Group 1. Feeding Mosquitoes.—Nov. 13, 1916. Adult mosquitoes hatched in the laboratory from the larvæ collected in Queens were allowed to feed on *Macacus rhesus* 1 on the 4th day of experimental poliomyelitis. 30 mosquitoes were well engorged when examined the next morning. Used for experiments on Nov. 20, or in 7 days.

Group 2. Feeding Mosquitoes.—Nov. 15, 1916. Another lot of adult mosquitoes hatched in the laboratory from the same material were fed on the same monkey (No. 1) on the 6th day of the disease. The animal showed partial paralysis of the limbs. 23 well engorged females were obtained. Used for experiments on Nov. 20, or in 5 days.

Group 3. Feeding Mosquitoes.—Nov. 16, 1916. Another lot of adults from a similar source were fed on the same monkey (No. 1) on the 7th day of the disease. The animal was completely paralyzed. 17 engorged females were collected. Used for experiments on Nov. 20, or in 4 days.

Group 4. Feeding Mosquitoes.—Nov. 7, 1916. A lot of adults hatched in the laboratory from the larvæ from Queens were fed on *Macacus rhesus* 2 on the 9th day of the disease. 25 engorged females were obtained. Used for experiments on Nov. 20, or in 13 days.

Group 5. Feeding Mosquitoes.—Dec. 4, 1916. A lot of adult mosquitoes hatched in the laboratory from the larvæ secured from a Jersey City suburb were fed on two *Macacus rhesus* monkeys, Nos. 3 and 4, on the 7th day of experimental poliomyelitis. 13 engorged females were obtained. Used for experiments on Dec. 19, or in 15 days.

Group 6. Feeding Mosquitoes.—Dec. 1, 1916. A lot of adult mosquitoes hatched in the laboratory from the materials gathered in the neighborhood of Jersey City were fed on *Macacus rhesus* 5 on the 3rd day of experimental poliomyelitis. 34 engorged females were obtained. Used for experiments on Dec. 15, or in 14 days.

Group 7. Feeding Mosquitoes.—Nov. 24, 1916. A lot of adult mosquitoes hatched in the laboratory from the material from Queens were fed on *Macacus rhesus* 6 on the 2nd day of the disease. 45 engorged females were obtained. Used for experiments on Dec. 15, or in 21 days.

⁸ Flexner, S., and Amoss, H. L., The relation of the meninges and choroid plexus to poliomyelitic infection, *J. Exp. Med.*, 1917, xxv, 525.

Group 8. Feeding Mosquitoes.—Nov. 25, 1916. A lot of adult mosquitoes hatched in the laboratory from the material from Queens were fed on *Macacus rhesus* 7 on the 5th day of the disease. The animal had not yet become paralyzed. 20 engorged females were collected. Used for experiments on Dec. 15, or in 20 days.

The results of the experiments were negative, in spite of the fact that many females (about fifty) attacked the monkeys and became fully engorged with the blood sucked from them.

As in the earlier series of experiments, many of these engorged females oviposited and gave rise to a new generation of larvæ. It was easy to propagate them in the laboratory for an indefinite period. These offspring were in turn allowed to bite a normal monkey, but so far no positive transmission of the infection by their bites has resulted.

Experiments with Non-Biting Flies Reared in the Laboratory with an Abundant Quantity of Poliomyelitic Virus.

Non-biting adult flies may mechanically carry the virus of poliomyelitis just as they do various pathogenic microorganisms.⁹ But no experimental evidence has been adduced to prove whether the virus can penetrate into the interior of the fly larvæ when the latter are fed on the poliomyelitic material, or, if it does, whether it is capable of multiplication within the body cavity of the larvæ. The question here is not the same as that of blood-sucking flies or mosquitoes, which may be the intermediary hosts as well as the direct transmitters of the virus by their bites. But in the case of non-biting flies, though the virus may multiply in their bodies, its distribution must depend upon their well known habits of gathering about food or persons while they regurgitate, resuck, and excrete. In this way the question of the non-biting flies as intermediary hosts of the virus would be quite as important as that of the biting varieties.

In the present experiment, a number of young larvæ of house-flies (*Musca domestica*) and of bluebottle flies (*Calliphora vomitoria*) were fed with the brain tissue of a *Macacus rhesus*, which had died of ex-

⁹ Flexner, S., and Clark, P. F., Contamination of the fly with poliomyelitis virus. Tenth note, *J. Am. Med. Assn.*, 1911, lvi, 1717.

perimental poliomyelitis with typical symptoms and pathological findings. The activity of the material used was established in a subsequent experiment.

Group 1.—Nov. 23, 1916. About 50 house-fly larvæ were put on several slices of the fresh poliomyelitic monkey brain (No. 1). The larvæ eagerly penetrated into the nervous tissue as if trying to shield themselves from the light. At room temperature the tissue underwent a putrefactive decomposition within 24 hours, so the larvæ had to be placed on a fresh lot of the virus-containing brain. When left in the decomposed mass of tissue, many larvæ died. After being fed on the poliomyelitic brain for about 6 days, the larvæ became pupæ. After pupation, they were left with the brain tissue for several days longer and then washed from the tissue and removed to the refrigerator.

Group 2.—Nov. 28, 1916. Another lot of house-fly larvæ were similarly fed with poliomyelitic brain emulsion (No. 1) for 6 days. Pupation took place on Dec. 4. The pupæ were washed and removed to the refrigerator on Dec. 9.

Group 3.—Nov. 23, 1916. 25 larvæ of bluebottle flies were fed on several slices of brain tissue from a *Macacus rhesus* which had died of poliomyelitis. The larvæ attacked the tissue vigorously and within 24 hours seemed to have consumed a great deal of it. Unlike the house-fly larvæ, they feed on dead flesh or meat during their larval stage, but it was necessary to put them on fresh material every 24 hours in order to protect them from the deadly effect of the decomposed nervous tissue. Pupation occurred on Dec. 7. After several days, the pupæ were washed and removed to the refrigerator.

For the purpose of obtaining adult flies from the infected pupæ, a dozen specimens from each lot were put in a Petri dish and left at 80° F. Imagos hatched within about 12 days. These and the pupæ were used for the experiment described below.

*Preparation of Filtrate of the Infected Pupæ and Imagos of the
House-Fly and the Bluebottle Fly.*

70 house-fly pupæ, Groups 1 and 2.	}	Weight 1.36 gm.
12 " imagos, " 1 " 2.		
15 bluebottle fly pupæ, Group 3.	}	" 0.97 "
6 " " imagos, " 3.		

These were mixed together and thoroughly ground up in a mortar, 22 cc. of 0.9 per cent saline solution being added. The milky emulsion thus obtained was briefly centrifuged at a low speed to separate the coarse particles from the fluid. The latter was then passed through a sterile Berkefeld filter V, the result being a clear and sterile filtrate.

Animal Inoculation.—A *Macacus rhesus* was inoculated intracerebrally with 3 cc. of the above filtrate on Dec. 16, 1916. The result was negative, no symptoms suggestive of poliomyelitis having been observed.

The foregoing experiment indicates that it is improbable that the virus of poliomyelitis is taken up by fly larvæ and multiplies therein. Not only does the virus fail to multiply, but probably it is rapidly destroyed within the body of the insects. The notion that these non-biting flies may act as intermediary hosts or a virus reservoir is not justified by the evidence brought out in the present study.

SUMMARY.

1. *Culex pipiens* raised from the larval stage in water experimentally contaminated with an abundance of poliomyelitic virus were found to be incapable of causing the infection when allowed in large numbers to bite normal *Macacus* monkeys.

2. *Culex pipiens* which were fed on infected poliomyelitic monkeys during different stages of the disease were found to be incapable of transmitting the infection when allowed in large numbers to bite normal *Macacus* monkeys. A previous disturbance of the meninges by an injection of horse serum into the intrathecal space did not alter the result, which was negative.

3. The offspring of the mosquitoes which were either reared in the infected tanks or fed on infected monkeys were found to be entirely harmless when allowed to feed in large numbers on a normal monkey. There was no hereditary transmission of the virus from one generation to another.

4. No trace of the virus of poliomyelitis was demonstrable in the filtrate of an emulsion of adult flies and pupæ of the common housefly and bluebottle fly which were reared in the laboratory on slices, emulsion, or filtrate of monkey brain containing the poliomyelitic virus. The intracerebral injection of the filtrate produced no poliomyelitic infection in the normal monkey.

NEW ANAEROBIC METHODS.*

By WILSON G. SMILLIE, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

PLATE 8.

(Received for publication, March 12, 1917.)

The methods commonly used for the cultivation of anaerobic microorganisms are far from satisfactory. This applies to the partial anaerobes, such as the tetanus bacillus or *Bacillus botulinus*, but is particularly true of the absolute anaerobes, such as *Treponema pallidum* or the globose bodies of poliomyelitis.

The vacuum jar described by Noguchi¹ in his method for the cultivation of *Treponema pallidum* has proved satisfactory in his hands, but requires a very strong desiccator, accurately ground, and a special type of vacuum pump. The American desiccators which I have obtained during the past 2 years, however, have either been so poorly ground that they would not hold a vacuum, or else they were so poorly made that they collapsed when subjected to vacuum.

After many trials, my difficulties were finally overcome through utilization of the suggestions of Laidlaw,² who used a catalyzer of oxygen and hydrogen in the preparation of anaerobic culture tubes. McIntosh and Fildes³ developed the use of the catalyzer in making an anaerobic container, the "McIntosh bomb," but neither of these methods, useful as they are for the purposes for which they were devised, were suitable to our needs, and were therefore modified.

All the anaerobic methods to be described depend upon the catalytic action of platinized asbestos upon oxygen and hydrogen when they are brought into contact.

*This work was done under the tenure of a William O. Moseley Travelling Fellowship from Harvard University.

¹Noguchi, H., *J. Exp. Med.*, 1911, xiv, 99.

²Laidlaw, P. P., *Brit. Med. J.*, 1915, i, 497.

³McIntosh, J., and Fildes, P., *Lancet*, 1916, i, 768.

Methods for Anaerobic Test-Tubes.

The simplest method is used in the cultivation upon agar slants of such relative anaerobes as the tetanus bacillus or *Bacillus botulinus* and is similar to the method described by Laidlaw. Platinized asbestos is first prepared in the usual way, or it may be purchased from any laboratory supply house. A small mass of the catalyzer is firmly fixed at the end of a platinum wire by coiling the wire about it. The other end of the wire is inserted into a short glass rod, and the rod is inserted into a No. 1 one-hole rubber stopper. The apparatus is wrapped in a package and autoclaved.

The water of condensation is removed from a plain agar slant, the tube inoculated, inverted, the cotton plug removed, and the tube filled with hydrogen by means of a sterile capillary pipette. The hydrogen may be obtained from a Kipp generator, or more satisfactorily from a hydrogen tank. It should be passed through a series of wash bottles containing silver nitrate, sulfuric acid, potassium permanganate, and lead acetate to remove all impurities.

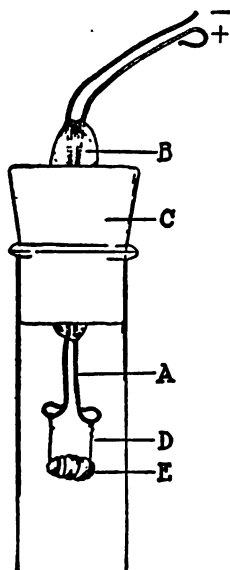
After allowing the hydrogen to fill the inverted inoculated test-tube, the platinized asbestos is heated for a moment in a free flame, the rubber stopper is inserted firmly into the inverted tube, and the end of the tube dipped into melted paraffin.

The catalyzer glows for a second or two as the hydrogen and oxygen are actively united, and the water formed is deposited upon the surface of the tube. The process is now complete, and the tube is ready for incubation.

This is essentially the method devised by Laidlaw and is very satisfactory for the growth of the usual anaerobes. The tetanus bacillus, for example, grows upon an agar slant in a thick, felt-like mass, in the profusion of its growth resembling a culture of *Bacillus subtilis*. It was necessary to devise a more strictly anaerobic method for our work, however, for Laidlaw's method could not always be relied upon to remove all traces of oxygen in the air, nor does it remove the oxygen from the surface of the media itself. The following method was devised to remove all the oxygen and has proved very satisfactory.

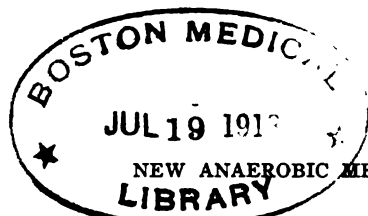
Two lengths of No. 22 nichrome wire, 6 cm. long, are separately

fused into a glass tube so that they are insulated (Text-fig. 1, A), and the glass tube, B, closed at each end, is passed through a one-hole rubber stopper, C. To the lower ends of the nichrome wire is attached a coil of fine (No. 31) nichrome wire, D, thus completing the circuit. In the coils of the fine wire is placed a small mass of platinized asbestos, E. The apparatus is placed in a package and autoclaved.



TEXT-FIG. 1. Sketch of anaerobic apparatus for the cultivation of absolute anaerobes in test-tubes.

A large test-tube, 20 by 1.5 cm. is used, to which 10 cc. of media are added, sterilized, and slanted. The water of condensation is removed and the tube inoculated. The tube is inverted, the cotton plug removed, and the tube filled with hydrogen by means of a sterile capillary pipette. The platinized asbestos mass, E, is heated for a moment in a free flame, and the rubber stopper, C, is then firmly inserted into the inverted tube and the end of the tube dipped in melted paraffin. The tube may now be placed in an upright position and sufficient electric current applied to the free ends of the wire to heat the fine nichrome wire wrapped about the platinized asbestos to a red heat. The catalyzer is thus heated, and the free



oxygen and hydrogen unite to form water. The tube is set aside for $\frac{1}{2}$ to 1 hour, then the platinized asbestos is reheated in order to ignite any residual oxygen. The tube may now be incubated.

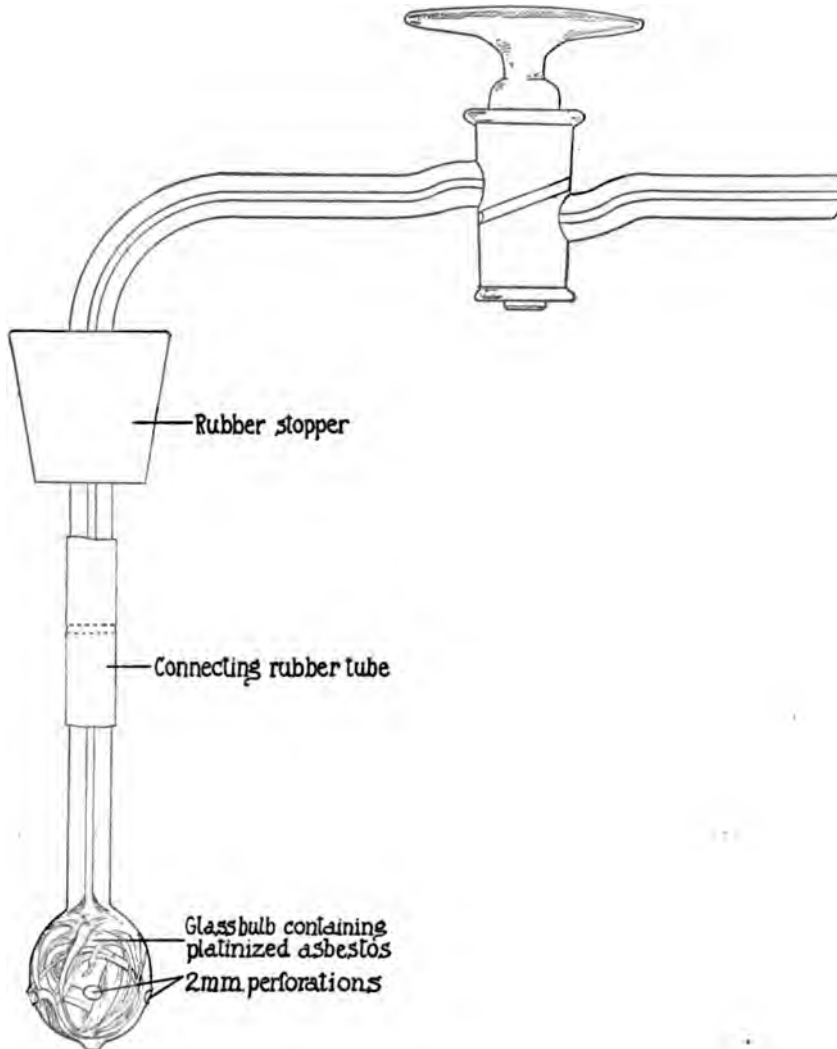
The method is very useful in growing all anaerobes, for the oxygen is always removed, whereas the Laidlaw method frequently fails. It is particularly useful for the cultivation of the stricter anaerobes. By this method the fusiform bacillus, for example, which is more strictly anaerobic than the tetanus bacillus, grows profusely in a thick, felt-like mass. By using this technique the organism of poliomyelitis grows upon ascitic agar slants, though much more slowly than other organisms; in fact, no growth is seen for 4 to 5 days, but at the end of 6 to 7 days, definite, tiny, raised, glistening colonies appear. These grow larger and larger, so that at the end of 12 to 14 days a definitely circumscribed, raised, opalescent colony is seen, some of these colonies even becoming as large as 1 mm. in diameter. In no instance was a growth of the globoid bodies of poliomyelitis obtained from the original material—brain and cord—but only from the fluid culture tubes of globoid bodies which had been growing in ascitic fluid media for several generations.

Method for an Anaerobic Jar.

The chief problem with which I was concerned, however, was the construction of an anaerobic jar, to be used as a container for a large number of tubes in the isolation of the organism of poliomyelitis from the infected brain and other tissues. All methods used for the cultivation of the globoid bodies failed in my hands until the jar described below was devised.

The jar used is an ordinary museum specimen jar (Fig. 1) about 30 cm. high and with an inside diameter of 12.5 cm. Two holes, 1.5 cm. in diameter, are ground in the cover, and into each hole is firmly inserted a No. 4 one-hole rubber stopper, carrying a ground glass "angle" stop-cock. To one of the stop-cocks is attached a rubber tube, at the end of which is a short piece of glass tube which reaches to the bottom of the jar. To the other stop-cock is attached, by a short rubber tube, a glass bulb, 2 cm. in diameter, which has been blown on the end of a capillary glass tube. The glass bulb is perforated with 5 to 6 holes, 2 mm. in diameter, and is filled with

platinized asbestos. The details of this apparatus are shown in Text-fig. 2.



TEXT-FIG. 2. Detail of the platinized asbestos bulb for the anaerobic jar.

Cultures are inoculated and placed in a glass tumbler, which is then placed in the jar, to which 100 cc. of a 10 per cent pyrogalllic acid solution have been added.

The glass bulb containing the platinized asbestos is heated over the free flame for a few seconds, and the cover is then cemented on. A rubber ring, 0.5 cm. thick, is placed between the jar and cover, all surfaces are cemented with Major's glass cement, and the metal clamp is screwed down with thumb and forefinger. The stop-cock to which the glass bulb is connected is placed on the vacuum pump, and gentle suction is applied for 2 to 3 seconds in order to insure a good initial flow of hydrogen and thus ignite the platinized asbestos at once. The stop-cock is now closed and attached to the hydrogen apparatus, and the gas is allowed to enter. This should be done carefully at first in order that an excess of hydrogen does not enter at once; for the gas should be burned as rapidly as it enters the jar. The platinized asbestos will soon be seen to glow and from this time hydrogen and oxygen will slowly unite, and the water formed will be deposited on the sides of the jar. When all the oxygen has united with the hydrogen, the platinized asbestos will become cool, but the hydrogen will continue to enter the jar until all the space formerly occupied by oxygen is replaced by hydrogen. The result is a hydrogen-nitrogen jar under approximately atmospheric pressure. The whole process should take about 15 minutes.

In order to have an index of the completeness of anaerobiosis the second stop-cock is connected with a bottle of 20 per cent sodium hydrate, freshly washed with hydrogen. By means of slight suction through the first stop-cock, 25 cc. of the sodium hydrate solution are drawn into the jar. Both stop-cocks are now closed, the ends sealed with cement, and the jar is incubated.

If the jar is satisfactory, the mixture of sodium hydrate and pyrogalllic acid will remain colorless indefinitely. This solution should not be relied upon to absorb any remaining traces of oxygen, but is simply an indicator of the presence of oxygen, and if it becomes discolored, there has been a mistake in technique, and the jar is unsatisfactory; therefore the cover should be removed, and the process repeated.

Method for Blake Bottles and Flasks.

The use of the platinized asbestos in a perforated glass bulb has been applied to mass cultures of anaerobes in flasks or Blake bottles.

The Blake bottle is useful when large amounts of an anaerobic organism, such as the tetanus bacillus, are desired, and is particularly useful when a differential anaerobic plate is desired as, for example, in the isolation of anaerobes from the pus of infected wounds.

A ground glass stop-cock of the usual type is inserted through a No. 3 one-hole rubber stopper. A heavy glass bulb, 1 cm. in diameter, is blown at the end of the stop-cock, and five or six small perforations are made in the bulb. The bulb is then filled with platinized asbestos, and the whole apparatus autoclaved.

The Blake bottle is inoculated, the platinized asbestos heated in a free flame, and the rubber stopper tightly inserted into the bottle. Slight vacuum is then produced in the bottle by gentle suction, in order to insure a good flow of hydrogen. The stop-cock is connected with the hydrogen generator and the gas allowed to enter. The catalyzer glows for a few minutes, then cools, and in 5 minutes the oxygen has been replaced by hydrogen, and the process is complete.

The use of the perforated bulb has not proved satisfactory in single test-tubes because of the small air space.

The methods are simple, rapid, clean, and efficient. They are not expensive, for only a small amount of platinized asbestos is needed, and the material may be used repeatedly without deterioration. It would at first appear that there might be some danger of explosion in the jar, but such is not the case. The hydrogen ignites as soon as it enters the jar so that there is never an excess of hydrogen in the container. As the hydrogen and oxygen unite to form water, a slight vacuum is formed and this vacuum insures a continuous gentle flow of hydrogen until all the oxygen has been replaced.

The precautions to be taken are as follows: (1) Allow the hydrogen to enter the jar slowly. (2) Be sure that the hydrogen is catalyzed as it enters, as evidenced by the glowing of the asbestos. After the glow has once appeared the remainder of the process will continue gently and completely. (3) Do not disconnect the apparatus while the process is taking place, for there is a slight vacuum in the jar which has only partially been replaced by hydrogen, and if air is allowed to rush in over the catalyzer, there is a possibility of a slight explosion.

The results that have been obtained by the use of these methods, particularly the anaerobic jar, will be published in a subsequent paper.

CONCLUSIONS.

1. Anaerobic methods have been devised which depend upon the catalytic action of platinized asbestos upon hydrogen and oxygen.
2. The methods may be utilized for the growth of anaerobes in test-tubes, upon Blake bottles, in flasks, and in a large container.
3. Because oxygen is so completely removed, the methods are of great value in the successful cultivation of absolute anaerobes.

EXPLANATION OF PLATE 8.

FIG. 1. Anaerobic jar with platinized asbestos bulb

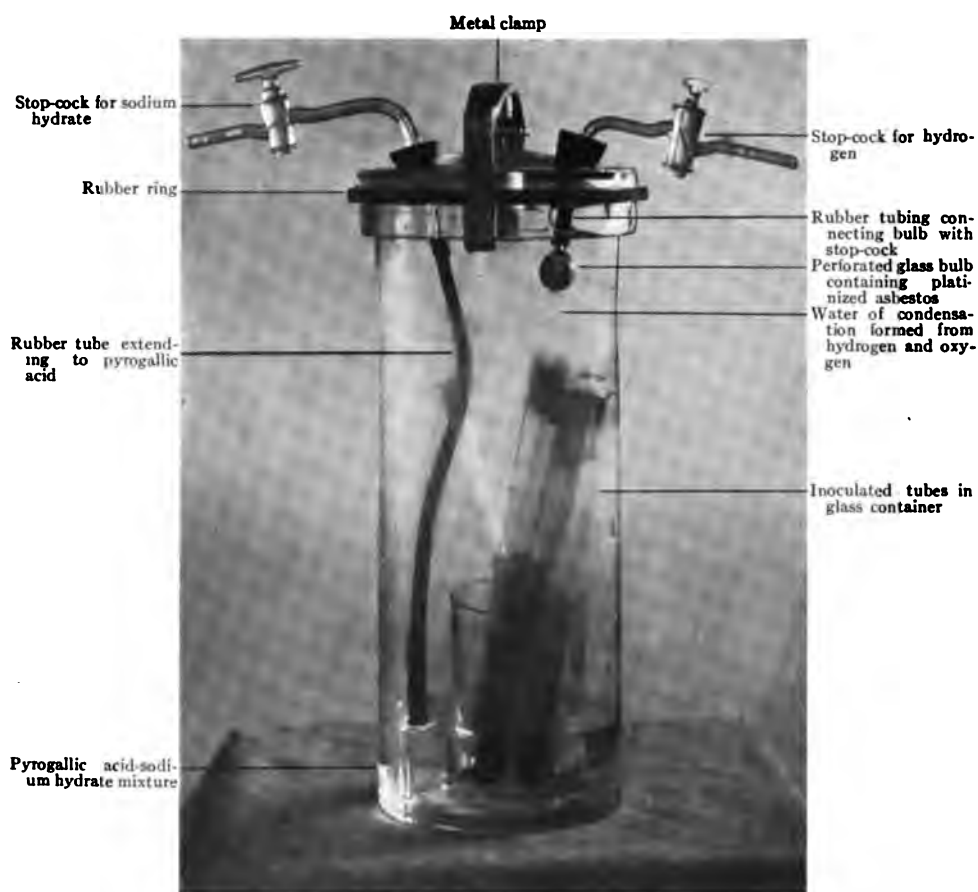


FIG. 1.

(Smillie: New anaerobic methods.)

TOXIN AND ANTITOXIN OF AND PROTECTIVE INOCULATION AGAINST BACILLUS WELCHII.

By CARROLL G. BULL, M.D., AND IDA W. PRITCHETT.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, April 15, 1917.)

This study of the pathology of infection by the group of *Bacillus welchii* has followed from several fortuitous circumstances. First, there was the insistent problem, only partially solved by the improvement in the antiseptic treatment of wounds, of gas bacillus infection following shell and bullet wounds everywhere in the war; second, there were available to us several cultures of *Bacillus welchii* isolated during the summer of 1916 on the western battle front by Dr. Simonds, who kindly presented them to the Institute; and finally and especially, Dr. Flexner's wish that with these cultures the subject of gas bacillus infection of the pigeon which he had observed many years before at the Johns Hopkins Hospital should be reinvestigated, as, in his opinion, the process in that species of animal epitomized the pathologic effects occurring in gas gangrene in man, and because he believed that a better understanding of the one condition would serve to explain many still obscure points in the other.

It will be of interest in this connection to review briefly certain facts concerning gas bacillus infection in the pigeon, since the condition is one little known to pathologists and bacteriologists. The classical article by Welch and Nuttall¹ on the gas bacillus appeared in 1892. It was followed by a paper on gas bacillus infection in man by Welch and Flexner² in 1896. The latter article was incomplete,³ and the concluding part which was to appear in the next number was never published. The second paper was to deal more particularly with experimental gas bacillus infection in animals—in the guinea

¹ Welch, W. H., and Nuttall, G. H. F., *Bull. Johns Hopkins Hosp.*, 1892, iii. 81.

² Welch, W. H., and Flexner, S., *J. Exp. Med.*, 1896, i. 24.

³ Personal communication from Dr. Flexner

pig and pigeon particularly. Since the pigeon had proved to be highly subject to infection and to respond with characteristic pathologic reactions, that animal came to be employed by the laboratory staff in the more or less routine study of the gas bacillus. But because of the circumstances stated, no full and sufficient description of the local lesions in the pigeon, which in disorganizing effect are comparable with the destructive lesions sometimes present in man, came to be published until some years later, when, at Dr. Flexner's suggestion, Dr. Herter,⁴ then engaged in the study of the *Bacillus welchii* group of bacteria occurring in the alimentary tract, employed this animal for inoculation. The lesions as described by Herter agree closely with those present in our pigeons inoculated with cultures of the bacilli.

Sources and Nature of Cultures.

The main part of our experiments has been made with five strains of *Bacillus welchii*, of which four were obtained through the kindness of Dr. Simonds. The fifth was isolated by us from a piece of clothing which had long been worn. The history of the Simonds cultures follows. The tests given were made by him.

Strain 365 a.—Isolated, Aug. 13, 1916, from scrapings from a bullet wound of the thigh, which showed a moderately severe gaseous gangrene. Its virulence for laboratory animals had not been tested.

Strain 386 cd.—Isolated, Sept. 9, 1916, from a fragment of shell with adherent bits of clothing, removed from a wound of the thigh. The patient did not develop gaseous gangrene. The organism had not been pathogenic for guinea pigs.

Strain 617 d.—Isolated, Aug. 27, 1916, from a case of violent gaseous gangrene following a bullet wound of the thigh with injury to and subsequent ligation of the large vessels of the leg. The limb was amputated. Although the stump was gaseous, the patient recovered. This strain is very pathogenic for guinea pigs, producing typical lesions and killing the animal in less than 24 hours after subcutaneous injection of 0.5 cc. of a 24 hour dextrose broth culture.

Strain 669 b.—Isolated, Aug. 21, 1916, from a case of gaseous gangrene following a bullet wound which caused shattering of the lower end of the femur. The leg was amputated; the patient recovered. Injection of 0.5 cc. of a 24 hour dextrose broth culture subcutaneously into a guinea pig was followed by a local-

⁴ Herter, C. A., Bacterial infections of the digestive tract, New York, 1907, 198.

ized gaseous gangrene and sloughing of the skin and subcutaneous tissues. The animal died 2 weeks after the injection. No gas bacilli were found at autopsy in films from tissues adjacent to the slough.

The history of our own strain is as follows: A piece of cloth from the lining of an old overcoat was thrust with tissue forceps deep into the right breast muscle of an anesthetized pigeon. The next morning the wounded muscle was greatly swollen, and crepitation was present. Pressure near the wound forced out gas bubbles which ignited with a snap. The pigeon was drooping and died at 2.30 p.m.

Autopsy.—The skin over the right side was edematous and covered with blebs. There was a reddish brown gelatinous exudate in the subcutaneous tissues of both groins and extending over the right pectoral muscles. A film preparation made from this exudate contained a few plump Gram-positive bacilli. The inoculated muscle was edematous and necrotic, most pronounced about the cloth, but extending along the muscle sheaths to the insertion of the fibers. Films from the necrotic muscle contained many plump Gram-positive bacilli and a few Gram-positive diplococci and Gram-negative bacilli.

Tubes of recently boiled litmus milk, blood agar, and blood bouillon were inoculated with the subcutaneous exudate and necrotic muscle. After 24 hours' incubation, the milk tubes showed the so called "stormy fermentation," and those inoculated with the subcutaneous exudate contained a plump Gram-positive bacillus in pure culture. The tubes from the necrotic muscle contained a similar bacillus, together with Gram-positive cocci in pairs and short chains and a Gram-negative bacillus. The blood media inoculated with the subcutaneous exudate remained sterile, while those of the necrotic muscle yielded cocci and Gram-negative bacilli only, the latter proving to be a variety of *Bacillus coli*. We shall call the culture of the Gram-positive bacillus, to be described more fully later, P-50.

The five cultures of Gram-positive bacilli enumerated have been tested for motility, spore formation, quantitative acid and gas production, liquefying action on gelatin at 22° and 37°C., pathogenicity for guinea pigs, rabbits, and pigeons, and still other properties. For example, the several strains have been tested for agglutination and lysis in normal rabbit and guinea pig sera, for hemolysins, for gas production in rabbits after the method of Welch and Nuttall, and for agglutination in artificially produced immune sera. As far as these tests are indicative, they place all five cultures among the group of *Bacillus welchii*; so far as they relate to specific properties, e.g.,

specific and cross agglutination, they indicate certain differences among them such as have commonly been observed among members of the group.

Pathologic Effect in Animals.

The feature of most importance among the properties just given which define this group of bacilli is pathogenic effect. This is true in the first place because of the similarity of certain of the lesions in animals to those arising in gaseous gangrene in man; and next and especially, because the investigation of the manner in which the lesions arise in animals led us to the discovery of the conditions under which a highly potent soluble toxic agent is regularly produced by the bacilli on which their poisonous or lethal action chiefly if not wholly depends.

In the past, the laboratory animal usually employed to test pathogenicity of *Bacillus welchii* has been the guinea pig. Many more cultures produce mild local infections, from which recovery takes place, than severer ones which are fatal. But the subcutaneous or intramuscular injection of cultures gives rise to local swelling, gas production, liquefactive necrosis of the involved muscle and skin, and, if the animal survives, eventual sloughing and cicatrization.

The rabbit is more resistant to local infection than is the guinea pig, but nevertheless lesions can be produced by active cultures which in general resemble those of the guinea pig. The rabbit reacts either to more highly virulent cultures or to larger doses of less effective ones. In the case of the former, death may result. But in both the rabbit and the guinea pig general blood invasion, even when the course of the infection is lethal, either does not take place at all or so few bacilli enter the blood that the cause of death cannot be attributed to a septicemia.

The peculiar susceptibility of the pigeon to infection with *Bacillus welchii* has not been utilized to the degree deserved. For in no other laboratory animal is infection produced so readily and with such a wide number of cultures, and in no other does the pathologic process proceed so swiftly and characteristically and with effects so nearly resembling the condition of gaseous gangrene in man. Probably this lack of use of the pigeon is to be ascribed to the rather brief refer-

ences made to the subject in the literature, which is almost confined to a few summary statements emanating from the Johns Hopkins Hospital at about the period already referred to. Dr. Flexner himself employed mainly blood containing large numbers of the bacilli for inoculating pigeons. In some instances the blood was taken post mortem from human cases, and in others from rabbits injected, killed, and incubated by the Welch-Nuttall method. Inoculation from pigeon to pigeon was also carried out. The animals succumbed at periods ranging from 5 to 24 hours. The autopsies revealed lesions precisely like those already described (page 121), except that the injected blood would often remain to tinge the tissues. The bacilli were very numerous in the disorganized muscle, fewer in the gelatinous exudate, and very few in or absent from the heart's blood.

The five cultures were pathogenic for pigeons, although not equally so. Simonds' Culture 617 d proved most virulent, not only for pigeons, but for rabbits and guinea pigs also. Doses of 0.01 to 0.05 cc. of a 20 hour glucose broth culture injected directly into the breast muscles were invariably fatal to pigeons in 6 to 20 hours. 0.0005 cc. of such a fluid culture put upon a bit of gauze and introduced into the muscles usually but not invariably produced fatal infection. Of Cultures 365 a and 669 b approximately 0.1 cc. of a glucose broth culture was the minimal lethal dose; while the dose of Cultures 386 cd and P-50 required to produce corresponding effects was 0.2 to 0.3 cc. The minimal lethal doses for guinea pigs and rabbits were several times those used for the pigeons.

Cause of Death in Bacillus welchii Infection.

In man, infection with *Bacillus welchii* tends to be a local process, even when severe, and invasion of the general blood occurs if at all only during the death agony or post mortem. In a small number of instances in man general infection seems to have played an important part in causing or hastening death.⁵ But as these cases are the exception, even when death occurs, in man as well as in the pigeon, rabbit, and guinea pig, it may be assumed that soluble chemical substances entering the circulation from the local lesion bring about

⁵ Thaon, P., *Compt. rend. Soc. biol.*, 1908, lxiv, 863.

the severe symptoms and the fatal termination. The question at once arising from this general consideration relates to the probable nature of the poisonous bodies. Several possibilities present themselves: They may arise from the bacilli, they may be yielded by the disorganizing tissues, or they may be of the nature of acids which disturb profoundly the hydrogen ion concentration of the body fluids. The protocols which follow are given to show the manner in which death may be produced in rabbits and pigeons under circumstances in which the blood remains wholly or practically free of the bacilli.

Experiment 1.—Pigeon. 11 a.m. 0.2 cc. of a glucose broth culture of Culture 617 d was injected into the breast muscle. 6.30 p.m. Animal died. Immediate autopsy. Usual gaseous, gelatinous, and necrotic local lesions containing myriads of bacilli. Films from the heart's blood and peritoneal fluid showed no bacilli, although a few were present, as a glucose broth culture was positive.

Experiment 2.—Pigeon. 11 a.m. 2 cc. of broth culture of Culture 617 d injected into breast muscles. 2.30 p.m. Animal died, having survived $3\frac{1}{2}$ hours. Local lesions similar to the preceding experiment, but the films from heart's blood contained a few bacilli.

These experiments were varied and repeated with different doses of the culture, but in no instance did a severe septicemia develop.

Experiment 3.—Rabbit. 11 a.m. Sedimented bacilli from 10 cc. of a 24 hour glucose broth culture of Culture 365 a were injected into the ear vein. 12 m. Films from the heart's blood showed a few bacilli, and the culture was positive. 9.30 p.m. Animal stuporous and breathing heavily. 10 p.m. Films and cultures from heart's blood negative. 10.20 p.m. Died. Immediate autopsy showed a dark and large spleen, but blood intact and serum clear. Films and cultures yielded no bacilli from the blood but some from the spleen and liver.

Experiment 4.—Rabbit. Mar. 14, 1917. 11 a.m. Sedimented bacilli from 2 cc. of broth culture No. 617 d given intravenously. Mar. 19, 8 a.m. Dead. Autopsy showed bloody fluid in peritoneum and small amount of clear fluid in pleura. The former contained many, the latter few bacilli; the heart's blood contained none.

It is apparent from these experiments that the death of the animals—pigeons and rabbits—is not closely bound up with the multiplication of the bacilli in the general circulation. The rabbit experiments indicate that the bacilli have no power to remain in the blood stream, and even when diminishing in numbers in the internal

organs still exert poisonous effects. Hence it may be concluded that poisons are liberated from the bacilli, but whether merely secreted or only yielded upon disintegration is not indicated by these tests. The next step therefore was to look for soluble toxic substances in the fluid cultures. For this purpose 24 hour glucose broth growths were employed.

Experiment 5.—(a) Rabbit; weight 1,950 gm. 8 cc. of Culture 669 b were injected intravenously. Immediately following the injection the animal became greatly excited, respiration became rapid, prostration followed, and death occurred in 7 minutes from respiratory failure.

(b) Rabbit; weight 1,700 gm. 9 cc. of Culture 617 d similarly injected. 14 minutes later this animal suddenly became excited, jumped from the basket, and died 7 minutes later of respiratory failure.

(c) Rabbit; weight 1,800 gm. 10 cc. of Culture 365 a intravenously injected. Died within 6 minutes under similar circumstances to those in (a) and (b).

In other words, an acutely fatal effect can be produced from large quantities of a broth culture injected intravenously. Less quantities (1 to 2 cc. per kilo) produce no immediate symptoms but cause death in from 6 to 24 hours. Differences in virulence only appear from the smaller doses and at once distinguish Culture 617 d as the most active. The massive doses of cultures exerted an injurious effect on the red blood corpuscles, which are destroyed in large numbers. On the other hand, the bacilli do not tend to agglutinate rapidly in the blood stream. By performing *intra vitam* agglutination tests, it was found that Culture 386 cd alone became rapidly agglutinated. This one was the least active, requiring the largest dose to cause acute death. The blood became free of the other cultures only after 4 to 8 hours. It appears then that bacillary embolism is not the probable cause of the acute lethal effects.

The toxicity of the fluid portion of the broth cultures was investigated. Since in these tests centrifugation alone was employed, pigeons could not be used, as the small number of bacilli remaining was sufficient to cause infection; hence rabbits were again injected. We may mention here that the injection of 2 to 4 cc. of the centrifuged fluid subcutaneously or intramuscularly into rabbits would sometimes lead to severe local and fatal infection. The fluid therefore possessed aggressive activity.

Experiment 6.—(a) Rabbit; weight 1,475 gm. Injected 10 cc. of supernatant fluid of glucose broth culture No. 617 d intravenously. Immediate collapse, air hunger, and death in 2 minutes. The red corpuscles were largely disorganized. The serum was reddish brown, and the remaining corpuscles appeared as mere shadows.

(b) Rabbit; weight 1,400 gm. Before injection the red corpuscles numbered 5,600,000 and the white 8,700 per c.mm. 12 m. 4 cc. of supernatant fluid of Culture 617 d were injected intravenously. 20 minutes later breathing was rapid and labored; the animal was very weak, and the red cells had fallen to 80,000 per c.mm. The white cells were unchanged. 1.20 p.m. Animal prostrate, the red cell count 84,000, and the white cells 8,300 per c.mm. 10 minutes later respiration ceased. The kidneys were dark brown in color, the bladder contained dark brown urine, and the few remaining red corpuscles were shadowy.

(c) Rabbit; weight 1,375 gm. Red cells 5,250,000 per c.mm. 11.50 a.m. Received 3 cc. of supernatant fluid, Culture 617 d. 12.15 p.m. Respiration accelerated, red cells 1,600,000. 3 p.m. Red cells 1,650,000. Died in night. The urine found was dark brown in color. Kidneys chocolate colored.

It would appear to be shown by these experiments that the acutely fatal effects of massive doses of the broth cultures as such or when separated in large part from the bacilli themselves are due to some body causing rapid and extensive blood destruction. Whether any other factor plays a part these experiments do not determine.

The next experiments were devised to rule out the factor of acidity. The different supernatant fluids exhibited acidities ranging from 2.5 to 4.5 per cent in terms of normal sodium hydroxide, with phenolphthalein as indicator. References in the literature point to the acid and especially the butyric acid content of cultures as responsible for the toxic effects. Two sets of tests were made (1) by neutralizing the broth with sodium hydroxide and (2) by comparing the acidity with the toxicity of different fluids.

Experiment 7.—Rabbit; weight 1,575 gm. Red cells 5,450,000. 12 m. 5 cc. of supernatant fluid of Culture 617 d neutralized with sodium hydroxide were injected intravenously. 12.35 p.m. Red cells 1,600,000. 1.50 p.m. Red cells 800,000. Respiration rapid, animal tires easily. 3 p.m. Red cells 600,000. Animal died during night. The autopsy showed the dark urine and chocolate colored kidneys as described above.

The exclusion of the acidity in the fluid may diminish somewhat the intensity of the blood destruction but does not remove it or

prevent the fatal issue. Experiments similar to this were made a number of times with consistent results. Moreover, comparison of toxic action and the degree of acidity was made, from which it was seen that acidity and lethal effects do not proceed hand in hand.

Experiment 8.—(a) Rabbit; weight 1,400 gm. Red cells 5,700,000 per c.mm. 2.45 p.m. 8 cc. of supernatant fluid of Culture 617 d, having an acidity of 1.9 per cent normal sodium hydroxide, were given intravenously. 4.45 p.m. Red cells 1,240,000. 5 p.m. Animal breathing rapidly; very weak. Died during night. Usual autopsy findings.

(b) Rabbit; weight 1,600 gm. 2 p.m. 10 cc. of supernatant fluid of Culture 386 cd, having an acidity of 4.5 per cent normal alkali, were injected intravenously. No symptoms appeared, and the red corpuscles were only slightly reduced in number. The animal still lives.

These experiments show that the acidity is not the main factor in causing either blood destruction or the fatal effects, and they are supported by tests on pigeons, examples of which follow; they indicate also that *Bacillus welchii* produces, in the test-tube at least, an active hemolysin.

Experiment 9.—(a) Pigeon. Red cells 4,645,000 per c.mm. 9.50 a.m. 0.5 cc. of 24 hour glucose broth culture No. 365 a injected into wing vein. 10.40 a.m. Red cells 4,700,000; no free nuclei present. 11.50 a.m. Pigeon drooping. Red cells 3,880,000, free nuclei appearing. 2 p.m. Red cells 2,432,000; many free nuclei. 3.45 p.m. Dying. Red cells 1,520,000. Immediate autopsy showed some but not numerous bacilli in blood; cytoplasm of the red cells stains weakly; blood serum reddish brown in color.

(b) Similar to (a). In 8 hours, when death occurred, the red cells had fallen from about 5,000,000 per c.mm. to 1,000,000. The autopsy findings were typical.

In other words, the injection of 0.5 cc. of a broth culture of active *Bacillus welchii* intravenously into pigeons causes extensive blood destruction and death in periods of from 6 to 8 hours. These results are now to be contrasted with pigeons in which the culture is injected into the pectoral muscles.

Experiment 10.—(a) Pigeon. Red cells, 4,500,000 per c.mm. 9.30 a.m. 0.5 cc. of glucose broth culture No. 365 a injected into breast muscle. 12 m. Drooping. 3.30 p.m. Red cells 4,600,000; no free nuclei. 4.45 p.m. Dying. Red cells 4,450,000. Autopsy showed local infection; no bacilli in blood.

(b) Similar to (a) except that Culture 617 d was employed. At the outset the red cell count was 4,450,000 per c.mm. 6 hours later, when the animal was dying, it was 4,435,000. The autopsy findings were characteristic.

These experiments several times repeated were always consistent. Intravenous injections of broth cultures are attended by extensive blood destruction and death; intramuscular injections of like doses cause death with equal certainty and rapidity but no blood destruction. Hence the blood destruction cannot be the determining factor of the lethal action. The essential toxic agent appears now not to be an acid and not an hemolysin. The next experiments relate to its filterability.

Up to the present the fluid cultures described were not wholly free from the bacilli. To remove the bacilli entirely, in order to test the toxicity of the sterile fluid, filtration through a Berkefeld N candle was resorted to. The first tests were made with filtrates obtained from ordinary glucose broth cultures. They indicated merely a low degree of toxicity for rabbits and pigeons.

Experiment 11.—A 24 hour glucose broth growth of Culture 617 d was employed as follows:

(a) Rabbit; weight 1,475 gm. 10 cc. of the supernatant fluid obtained by centrifugation for 20 minutes, not quite clear, and having an acidity of 4.2 per cent were injected intravenously. The animal had a severe convulsion and died almost immediately. The blood was extensively destroyed.

(b) Rabbit; weight 1,425 gm. 11 cc. of a Berkefeld filtrate, having an acidity of 3.9 per cent, were injected intravenously. No symptoms.

(c) Rabbit; weight 1,575 gm. 3 p.m. 10 cc. of the clear fluid obtained by centrifugation for 40 minutes and having an acidity of 4.5 per cent were injected intravenously. For an hour there was respiratory distress, which passed off. 7 p.m. Died. Kidneys chocolate colored. Another part of this fluid first filtered, then injected into a normal rabbit, produced no effect.

This experiment shows that not only does filtration reduce the toxicity, but long centrifugation does also. The difference is not caused by the reduction in acidity observed in the filtered fluid, since neutralization of the centrifugate with sodium hydroxide did not affect its activity. The injection of the filtrate in amounts of 8 cc. into the breast muscles of pigeons caused temporary drooping but no local lesion or other severe effect. No distinction in action was

noted in the different cultures. The conclusion to be drawn from these experiments is that a certain kind of toxic product is developed in glucose broth cultures, but that prolonged centrifugation and filtration tend to remove it from the fluid. Since the action is so rapid, it does not seem probable that there is any actual relationship between the bacilli as such still remaining in the centrifuged fluid and the poisonous agent. The latter is not an ordinary acid and appears to be an hemolysin.

The next experiments throw an entirely different light on the toxin-producing property of *Bacillus welchii*. It is obvious that cultures in glucose broth in no way represent the conditions occurring during local infections in man and animals. Hence these were simulated in the following manner.

To plain beef infusion broth in 10 cc. quantities in test-tubes were added several fragments of sterile skeletal muscle of the pigeon or rabbit. The tubes, having been proved sterile, were inoculated with *Bacillus welchii* and overlaid with paraffin oil and enclosed in a vacuum jar from which the oxygen was exhausted. After an incubation of from 18 to 24 hours, the fluid was centrifuged and filtered through a Berkefeld N candle. The filtrate was always free of the bacilli. This product proved highly toxic for pigeons, guinea pigs, and rabbits, and, what should be emphasized, gave rise to inflammatory and other local lesions resembling closely those caused by the bacilli themselves. While Culture 617 d, the most virulent of all, yielded the most active filtrate, yet all five cultures gave toxic products. The degree and manner of the action of the toxic filtrates are indicated by the following illustrative protocols.

Experiment 12.—(a) Guinea pig. 3 p.m. 2 cc. of the Berkefeld filtrate of an 18 hour pigeon muscle broth culture of Culture 617 d were injected beneath the skin of the right thigh. The next morning at 8 a.m., the entire leg was swollen and the joints held stiffly; the scrotum was also edematous. The animal crouched in the corner of the cage. 3 days later the hair was loose, and the tissues were sloughing. Death occurred during the night. The autopsy showed disorganization of the muscles of the right leg and adjacent abdominal wall. Cocci, but no gas bacilli were present.

Doubtless the filtrate acted upon the skin and underlying muscles, inducing inflammation and necrosis, after which pyogenic cocci and

other bacteria entered the injured tissues and produced the sloughing. A dose of 1 cc. of the filtrate caused a similar but less severe lesion, from which the guinea pig slowly recovered after healing of the defect.

(b) Rabbit. 2 cc. of the same lot of toxin used in (a) were injected under the skin of the right thigh at 4.30 p.m. The next day the skin over the leg and the right scrotal sac was highly edematous. 5 days later, the edema subsided, leaving a dry necrotic area behind, which finally was thrown off and became healed.

The local effect, therefore, in the rabbit is similar to although less severe than that in the guinea pig. The effect in the rabbit of an intravenous injection of the same lot of toxin is to produce acute blood destruction and death. Thus a rabbit having a red cell count of 5,400,000 was given 1 cc. of the toxin at 10 a.m. At 11 a.m. the cells numbered 4,250,000; at 12.30 p.m., 2,550,000; at 4 p.m., 1,500,000; at 5 p.m., 1,000,000. Death took place during the night. The kidneys were chocolate colored and the urine dark.

(c) Pigeon. 12 m. 0.2 cc. of a similar toxin was injected into the right breast muscles. The next morning at 8 o'clock there was widespread edema of the injected site. 24 hours later the edema was subsiding. 3 days later the swelling had disappeared. On etherization and autopsy, an extensive necrotic focus was found in the injected pectoral muscles; the muscles of the opposite side were normal.

The local reaction of the pigeon to small doses of the toxic filtrate resembles that of the guinea pig to far larger doses. When 0.3 cc. of the filtrate was injected, the edema developed very quickly and death occurred in about 4 hours—even more quickly, therefore, than from massive bacillary infection. The injected muscle was already friable. No effect is produced in the red blood corpuscles. When, however, the filtrate is injected into the wing vein, hemolysis results. Thus a pigeon having a blood count of 4,280,000 was given 0.25 cc. of neutral filtrate at 9.30 a.m. At 10.30 a.m., the cells were unchanged in number and no free nuclei occurred. At 4.30 p.m. the cells numbered 3,725,000, and free nuclei were found. The next day at 1 p.m. the cells numbered 800,000, and there was marked air hunger. Death took place at 5 p.m.

This experiment, which was repeated several times, shows that *Bacillus welchii*, when the conditions of growth are suitable, yields toxic products of high potency. These products produce two sets of effects according to the manner of their injection into animals: (a) hemolysis, in which they resemble the effects arising from ordinary glucose broth cultures; (b) inflammation and necrosis of subcutaneous tissue and muscles, in which they resemble the effects produced by the bacilli themselves. Even moderate quantities of the toxic filtrate locally injected may also bring about rapid death of pigeons.

It is now possible to answer the question placed at the head of this section of our paper. The cause of death in *Bacillus welchii* infection is not a blood invasion of the microorganisms and not acid intoxication, but an intoxication with definite and very potent poisons produced in the growth of the bacilli in the tissues of the body. This poison is readily produced in broth in the test-tube in the presence of sterile non-denatured muscle. To obtain it in quantity only minimal quantities of glucose (0.1 per cent) should be added to the broth, and the incubation of the anaerobic cultures should not exceed 24 hours. The poison or toxin is a complex of an hemolysin and another poisonous body. The latter is the more toxic, since it may bring about death under conditions in which no blood destruction takes place.

The Toxic Product.

Thermolability.—The toxicity of the filtered fluid is destroyed by heating 30 minutes at 70°C. in sealed tubes and is greatly diminished by similar heating at 62°C. The fluid subjected to the latter treatment no longer causes death in pigeons, even when large doses are injected, although a degree of necrosis of the muscles still results. A test made to determine the point indicates also that the substances exerting the toxic effects do not dialyze through collodion membranes.

Antigenic Properties.—The next step taken was that of determining whether the toxic product would act as an antigen. Two sets of tests were made: (a) the setting up of active immunity of the pigeon; (b) the production of an antiserum in the rabbit.

The former is difficult to accomplish because of the necrosis caused even by sublethal injections into the pectoral muscles of pigeons. However, by giving three carefully graded injections at weekly intervals, the animals may be kept in fair condition. 1 week after the last injection, the pigeons bore two lethal doses of the toxic filtrate without reaction.

The latter is accomplished with less difficulty. Large male rabbits were employed. 2 cc. of a neutralized filtrate of Culture 617 d were injected beneath the skin of the inner aspect of the thigh. This was followed by edema involving the scrotum. The edema subsided in a few days, leaving the scrotal skin necrotic and dry. 10 days after the first injection a second one was given on the opposite side. The effects were the same as the first. A third injection of 3.5 cc. was given on the right side after a similar interval. No reaction followed. The rabbit was now bled and a series of neutralizations performed, as shown in Table I. The toxic filtrate was mixed with the serum from the immunized rabbit or normal rabbit and injected immediately into the pectoral muscles of the pigeon or subcutaneously into the rabbit and guinea pig.

The table shows that the blood of a rabbit which has received three injections of a toxic filtrate from a given culture is capable of neutralizing not only that particular filtrate, but the filtrate from four other cultures as well. The neutralization is effective against the filtrate obtained from several distinct cultures and for the three species of animals—pigeon, rabbit, and guinea pig—employed.

Moreover, the neutralization is not only for the toxic substance causing inflammation and necrosis of the local tissues, but also for the specific hemolysin contained in the filtrates. This is an important point, since it controverts the notion that the blood destruction results from acids produced in course of growth of the bacilli.

Experiment 13.—(a) Pigeon. Red cells 4,250,000; no free nuclei. 2.10 p.m. Injected into wing vein mixture of 1 cc. of toxic filtrate of No. 617 d and 1 cc. of normal rabbit serum. 3.40 p.m. Red cells 1,312,000; many free nuclei. 3.40 p.m. Death.

(b) Pigeon. Red cells 4,500,000. 11.45 a.m. Mixture of 1 cc. of toxic filtrate of No. 617 d and 1 cc. of immune rabbit serum injected into wing vein. 1.45 p.m. Red cells 4,264,000. 10 a.m. next day. Red cells 4,300,000. No symptoms appeared.

TABLE I.

Hr. of injection.	Animal injected.	Toxic product.		Mixed with immune or normal rabbit serum.		Local reaction.	Final result.
		Quantity.	Source.				
S. M.		cc.		cc.			
9	Pigeon.	1	617 d	0.5	Immune.	None.	Survived.
9	"	1	617 d	0.5	Normal.	In 2 hrs. extensive edema.	Died, 2.10 p.m.
9	"	3	365 a	1.0	Immune.	None.	Survived.
9	"	3	365 a	1.0	Normal.	In 3 hrs. extensive edema.	Died, 4.10 p.m.
9	"	3	669 b	1.0	Immune.	None.	Survived.
9	"	3	669 b	1.0	Normal.	In 4½ hrs. muscle greatly swollen.	Died, 1.45 p.m.
9	"	3	P-50	1.0	Immune.	None.	Survived.
9	"	3	P-50	1.0	Normal.	In 5 hrs. muscle greatly swollen.	Died, 2.30 p.m.
9	"	4	386 cd	1.0	Immune.	None.	Survived.
9	"	4	386 cd	1.0	Normal.	In 5 hrs. muscle swollen.	Died, 2.45 p.m.
10	Rabbit.	2	617 d	1.0	Immune.	None.	Survived.
10	"	2	617 d	1.0	Normal.	Extensive scrotal edema.	Recovered.
10	Guinea Pig A.	2	617 d	1.0	Immune.	None.	Survived.
10	" " B.	2	617 d	1.0	Normal.	Extensive swelling of leg and scrotum.	Necrosis. Died on 7th day.

Neutralizing Proportions.—The next experiment was designed to determine the minimal lethal dose of the toxic filtrate and the necessary neutralizing quantity of immune serum for that dose. This having been ascertained, an experiment was conducted to decide whether the neutralization took place equally in multiple proportions.

Pigeons were employed for the tests. The minimal lethal dose of the toxic filtrate employed proved to be 0.3 cc., from which death resulted in about 8 hours. The perfectly neutralizing quantity

of the immune serum for this dose was 0.2 cc. When mixed together and injected into the pectoral muscles no reaction followed. The minimal protective dose of the immune serum was much smaller; namely 0.05 cc. But with this dose considerable local reaction manifested itself.

The experiment with multiple proportions of toxic filtrate and immune serum was made with twenty-five doses of each. Hence 7.5 cc. of the toxic filtrate and 5.0 cc. of the immune serum were mixed and the entire volume was then injected into the breast muscles of each of two pigeons. No signs of intoxication developed, and aside from slight local edema in one of the pigeons, no symptoms whatever appeared. From this it was concluded that the toxic filtrate and antitoxic rabbit blood neutralized each other perfectly in multiples of the single doses. In this respect the two resemble the corresponding toxins and antitoxins of *Bacillus diphtheriæ* and *Bacillus tetani*.

Protective and Curative Properties.

The experiments described having clearly shown that the toxic products of the growth of *Bacillus welchii* exhibit antigenic activities and readily give rise to the formation of active antitoxic substances, the obvious next step was to determine whether the immune serum developed possessed protective and curative properties. Two sets of tests bearing on these questions have been made.

In one, vegetative bacilli have been injected into the breast muscles of pigeons mixed with or followed by the immune serum. The result is to prevent or reduce the pathogenic effects otherwise produced. Normal rabbit serum has no such power of control. These experiments will be published in detail later.

In the other, the object was to imitate conditions of natural infection in man with a view to preventing infection from arising. For this purpose, the bacilli were cultivated by Dunham's method so as to obtain spores. Bits of gauze were impregnated with the sporulating cultures and thrust into the breast muscle of the anesthetized pigeons with a small hemostat. The wound at once filled with blood and became sealed. Hence the conditions of a foreign body carrying active spores of the gas bacilli imbedded in muscle tissue

and protected from access of air as occurring in man were reproduced on a small scale. The test proved a severe one. Five pigeons were employed in a series. One only was treated with the immune serum, the other four serving as controls. This plan was adopted to remove the fallacy of an accidental survival of the treated animal. 2.0 cc. of the immune serum were injected, partly about the wound, partly in the opposite breast. The four inoculated but untreated pigeons developed typical local lesions and succumbed in 20 to 40 hours after inoculation. The treated animal never showed any local or general symptoms, survived, and the wound healed about the foreign body.

The experiments briefly reported in this section of the paper seem to possess considerable importance. They indicate, indeed, that in *Bacillus welchii* infection in nature the development of the spores into vegetative bacilli may be prevented by a protective inoculation of an antitoxic serum, and also that the vegetative bacilli may be deprived by such a serum of their toxic products, which now appear to be their real offensive instrument. We are confronted, therefore, not only with a new point of view regarding the manner of the pathogenic action of the Welch group of bacilli but also with a new means of combating their pathogenic effects.

DISCUSSION.

The experiments presented appear to admit of one interpretation only; namely, that the Welch bacilli, under suitable conditions of growth, produce an active exotoxin, to which their pathogenic effects are ascribable. The toxic product, moreover, acts upon the local tissues and the blood in a manner identical with the action of the cultures. With the toxic product animals may be immunized actively and yield an immune serum which neutralizes the toxin perfectly and in multiple proportion. The toxic bodies would seem to be at least two in number: one causing blood destruction, hence an hemolysin, and the other acting locally on the tissues and blood vessels, causing edema and necrosis and probably exerting general toxic action in addition. The part each plays in bringing about the lethal effect seems to be determined by the manner of inoculation: to bring out the

hemolytic action intravenous injection is indicated; to bring out the locally destructive action, subcutaneous or intramuscular injection is required.

This conception of the manner of pathogenic action of the Welch bacilli is totally different from any view previously held. It is true that others have attributed the general symptoms in *Bacillus welchii* infection to an intoxication; but the poisoning meant was one ascribed on the one hand to decomposition products of the infected tissues (E. Fraenkel) and on the other to ordinary endotoxin absorption (Metchnikoff, Korentchewsky, Kamen, Herter, Passini). Other views have also been expressed and insisted upon, and they would ascribe the locally destructive effects of the bacilli to mechanical action (Taylor) or to the production of fatty acids which also through the setting up of an acidosis bring about a lethal termination (McCampbell, Stewart and West, Wright).

Reference will be made only to the views expressed by recent writers who have encountered gaseous gangrene in connection with gun-shot wounds of the great war. Thus Weinberg,⁶ who believes that the gas-producing bacilli do not cause the gangrene, but that the condition precedes the infection, has obtained toxic and antitoxic products from various anaerobic bacteria isolated from gangrenous wounds. With *Bacillus perfringens* (of the *Bacillus welchii* group) he has prepared an antibacterial serum, but he failed to detect either the exotoxin or its corresponding antiserum.

Kenneth Taylor⁷ considers that gaseous gangrene is the result of the mechanical action of the gas produced in a local focus of developing saprophytic bacteria. He specifically draws the distinction between *Bacillus tetani* and *Bacillus welchii* infections, since with the former the toxin is the active factor, while with the latter the mechanical effect of the gas is paramount. The mechanical process he conceives to be as follows: *Bacillus welchii* attacks the carbohydrates of muscular tissue and produces a large volume of gas, which, being unable to escape from the tissues, exerts pressure upon the blood vessels, impeding the circulation so that necrosis results. The necrotic tissue is invaded by putrefactive bacilli which disorganize it.

Sir Almroth Wright⁸ holds that *Bacillus welchii* operates through the production of an acid condition of the blood and tissues, through which the antitrypsin is diminished. Because of this diminution, tryptic digestion of the proteins is permitted and the bacilli are thus provided with a highly favorable medium of growth, so that multiplication becomes explosive in nature. The intoxication following is in fact an acidemia.

⁶ Weinberg, M., *Proc. Roy. Soc. Med.*, 1916, ix, Occas. Lect., 119.

⁷ Taylor, K., *Bull. Johns Hopkins Hosp.*, 1916, xxvi, 297; *J. Path. and Bacteriol.*, 1916, xx, 384.

⁸ Wright, A. E., *Proc. Roy. Soc. Med.*, 1916-17, x, Occas. Lect., 1.

Conradi and Bieling⁹ distinguish two phases of action of the bacilli. In the first or fermentation phase, the carbohydrates are attacked, and lactic, butyric, propionic, and succinic acids are formed, which are the immediate causes of the edema and necrosis of the tissues. In the second or saprophytic stage, the spore-bearing organisms appear and appropriate the dead tissue, giving rise to putrefaction and consequent intoxication.

These brief extracts readily indicate not only the wide diversity of opinion held by recent students of the pathogenesis of gas bacillus infection in man, but show also how remote the conceptions are from that of a specific pathogenetic process, due to the action of particular toxic substances, which is the basis of the conviction derived from the experiments described by us. According to our view, infection by *Bacillus welchii*, like infection by *Bacillus tetani*, essentially resolves itself into an intoxication, in which an exotoxin yielded by the multiplying organisms constitutes the chief danger. The two conditions differ, however, with respect to the local effects produced on the tissues, since the tetanus toxin does not possess inflammatory and necrotizing properties. The Welch bacilli, therefore, grow more abundantly and produce wide destruction of tissue, in which process they are soon assisted by the usual pyogenic microorganisms, which quickly obtain a foothold in the disorganized structures.

SUMMARY.

Five cultures of *Bacillus welchii* have been studied and compared. Four came from infected wounds in the western theatre of war, and one was obtained from a personal article of clothing. Each culture possesses the essential characteristics ascribed to that group of bacteria.

The infectious processes caused by the five cultures in rabbits, guinea pigs, and pigeons, are local in character; and very few or no bacilli enter or are found in the general blood stream during life or immediately after death.

Glucose broth cultures, injected intravenously, are fatal to rabbits. Death occurs almost immediately or after a few hours. Agglutinative bacterial emboli have been ruled out as the cause of death.

⁹ Conradi, H., and Bieling, R., *Münch. med. Woch.*, 1916, lxiii, 1608.

as has been an acid intoxication. The fluid part of the culture acts in the same manner as the full culture and irrespective of neutralization with sodium hydroxide.

The full cultures and supernatant fluid are hemolytic when injected directly into the circulation of rabbits and pigeons, and the acute death produced may be ascribed to a massive destruction of red corpuscles. The passage of the fluid portion of glucose broth cultures through Berkefeld filters reduces materially the hemolytic and poisonous effects.

Cultures of the Welch bacilli in plain broth to which sterile pigeon or rabbit muscle is added are highly toxic, and the toxicity is not noticeably diminished by Berkefeld filtration. The filtrates are hemolytic when injected intravenously and inflaming and necrotizing when injected subcutaneously and intramuscularly. The local lesions produced in the breast muscles of the pigeon closely resemble those caused by infection with the bacilli.

The toxicity of these filtrates is not affected by neutralization with sodium hydroxide, but is materially reduced by heating to 62°C. and entirely removed by heating to 70°C. for 30 minutes.

Successive injections of carefully graded doses of this toxic filtrate in pigeons and rabbits give rise to active immunity. The blood taken from the immunized rabbits is capable of neutralizing the toxic filtrate *in vivo* and *in vitro*. The filtrate has therefore been designated as toxin and the immune serum as antitoxin.

The antitoxin neutralizes the toxin in multiple proportions. Hence the latter would seem to possess the properties of an exotoxin. Moreover, it neutralizes the hemolytic as well as the locally injurious toxic constituent.

Antitoxic serum prepared from a given culture of *Bacillus welchii* is neutralizing for the toxins yielded by the other four cultures of that microorganism.

The antitoxin is protective and curative against infection with the spore and the vegetative stages of *Bacillus welchii* in pigeons. The limits of the protective and curative action are now under investigation.

THE EFFECTS OF EXPERIMENTAL PLETHORA ON BLOOD PRODUCTION.

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Our present knowledge concerning normal blood production indicates that the bone marrow functions at a constant rate supplying new red corpuscles to replace those lost daily in the process of normal blood destruction. Since an extra loss of blood causes greater activity of the bone marrow,¹ it has seemed possible that were the constantly occurring normal loss compensated for artificially there would be no necessity for the production of new cells, and consequently a lessened activity of the bone marrow might result. In an attempt to bring about this condition, rabbits have been made plethoric by repeated small transfusions of blood and the effect of the procedure on the bone marrow has been observed.

Some work has already been done in this direction. Hess,² with the purpose of determining the effect of plethora on the heart, gave rabbits repeated large transfusions. At the end of 2 months of plethora the rabbits were killed. In examining the bone marrow, he claimed to have found evidence of a markedly diminished activity. The erythroblasts and myelocytes were decreased in number, and there was a fibrous hyperplasia present. Itami³ gives a more detailed account of these findings, agreeing with Hess in his conclusions. Boycott and Douglas⁴ carried on a similar series of experiments but failed entirely to confirm Hess's and Itami's results, which seem to have been obtained in only a few animals.

¹ The term "bone marrow activity," as used in this paper, refers only to the erythropoietic function of the marrow.

² Hess, R., *Deutsch. Arch. klin. Med.*, 1909, xcv, 482.

³ Itami, S., *Folia haematol.*, 1908, vi, 425.

⁴ Boycott, A. E., and Douglas, C. G., *J. Path. and Bacteriol.*, 1909, xiii, 414.

The criteria which these two sets of investigators used as evidence of depressed bone marrow activity, that is, marked histological changes in the marrow, may account to some extent for their contradictory results, since the red marrow of even normal rabbits varies much in its morphology as indicative of blood production and also in its distribution.

In the present work, use has been made of an indicator which may be thought to represent more nearly the actual functional variations in the marrow taken as a whole; namely, the number of reticulated red cells in the circulating blood. In addition, the number of these cells in the bone marrow itself has been determined. In normal animals, reticulated red corpuscles are present in the blood in fairly constant numbers. There is good evidence that increased activity of the bone marrow is accompanied by an increase in the percentage of these cells, and that the percentage roughly parallels the degree of hyperfunction.^{5,6} *A priori*, one would suppose that if the activity of the bone marrow were lessened, a drop in the reticulated cell count would result.

Method of Producing Plethora.

The method of producing the plethora has already been described⁷ but will be briefly given here. Rabbits were used. Each recipient was provided with three to six donors of the same hemolytic group. Transfusions of 10 cc. of blood were made daily. The donors were used in rotation, so that each one lost at most only 10 cc. every 3 days. In this way the production of any considerable anemia in the donor rabbits was avoided. In some of these rabbits, the blood loss was made up so promptly that the hemoglobin percentage did not vary throughout the period of the bleedings; in others it dropped slightly. The blood for transfusion was obtained from the donor by cardiac aspiration into a syringe containing 1 cc. of a 1 per cent solution of sodium citrate in normal salt solution. This small amount of citrate, when well mixed with 10 cc. of blood, was sufficient to prevent coagulation for the few minutes required to introduce the blood into the marginal ear vein of the recipient. The recipient rabbits for the most part weighed from 1,500 to 1,800 gm. All were young rabbits. Both sexes were employed.

⁵ Lee, R. I., Minot, G. R., and Vincent, B., *J. Am. Med. Assn.*, 1916, lxvii, 719

⁶ Vogel, K. M., and McCurdy, U. F., *Arch. Int. Med.*, 1913, xii, 707.

⁷ Robertson, O. H., and Rous, P., *J. Exp. Med.*, 1917, xxv, 665.

The Sahli hemoglobinometer was used for determining the degree of plethora. The inaccuracies of this method are recognized, but dependable readings were obtained by diluting for the color comparison after the test mixture had stood exactly 5 minutes. In some of the animals, the red cells were counted and the color index was estimated.

Method of Counting Reticulated Cells.

The following method for counting the reticulated cells was found to be preferable to those previously described. A saturated solution of brilliant cresyl blue was made up in normal salt solution. This was kept as a stock solution. When a count was to be made, a small quantity of it was diluted 80 times⁸ with normal salt solution and mixed with blood in a pipette for counting white cells in the proportion of one part of blood to twenty parts of cresyl blue solution. The mixture was shaken in the pipette for 5 minutes. The cells were thus equally distributed as well as stained. They were counted at once in fresh preparations, which were sealed with vaseline to prevent disturbances due to drying. At least 1,000 red cells were counted at each test. When the number of reticulated cells was less than 1 in 1,000, 10,000 red cells were counted. In the latter case, only the first 1,000 were counted individually, the field being the unit of count for the remaining 9,000.

For several days before transfusions were begun, the number of reticulated cells was determined daily in those rabbits destined to be recipients.

In a large number of normal rabbits examined, the reticulated cells were found to vary for the most part between 10 and 20 per 1,000 erythrocytes. Rarely they were as many as 30 or fewer than 5 per 1,000. Only two animals showed less than 5 per 1,000, one having a count of 3 and the other of 4. The variation in the individual from day to day may be slight or, relatively speaking, considerable.

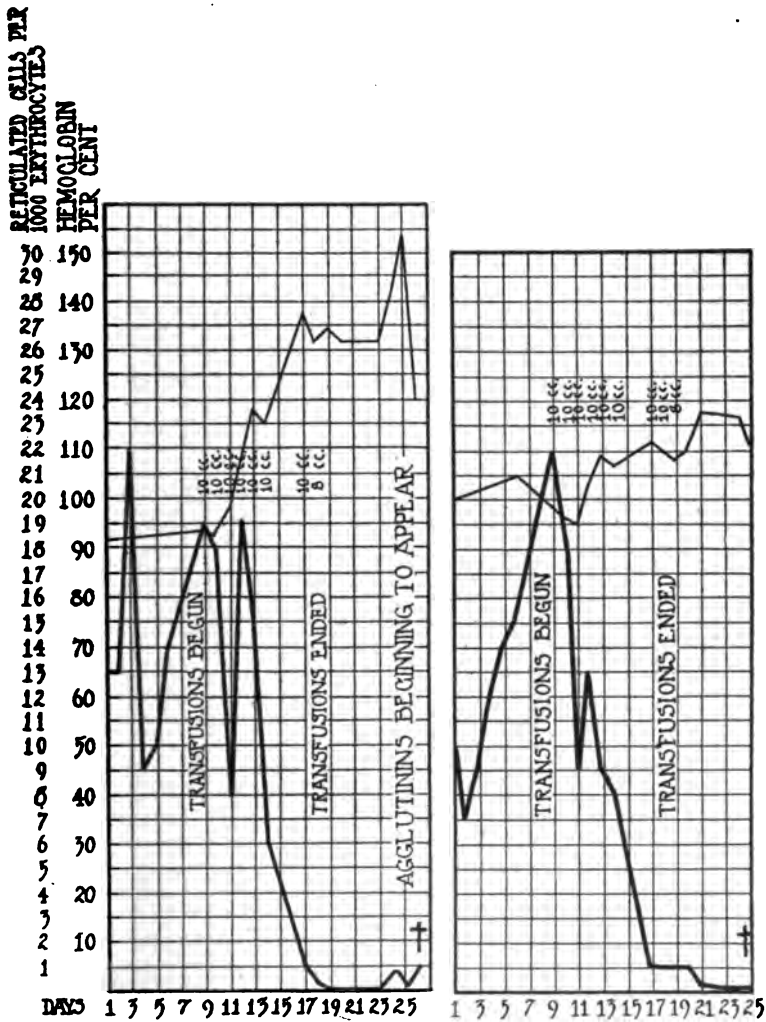
⁸ Since doing this work, a second saturated solution of cresyl blue has been made up, using a different stock of the dye, which went into solution to a considerably greater extent than the first. The result was that a 1:80 dilution of this saturated solution was much too strong a staining fluid. It was found necessary to dilute to 180 for satisfactory staining. It is apparent, therefore, that each saturated solution of cresyl blue has to be tested beforehand for its optimum staining dilution. This is a very simple matter and needs to be done only once.

Production of Plethora.

Sixteen rabbits were rendered plethoric by the method described. The rate at which the hemoglobin per cent increased in different animals varied considerably. In some it rose rapidly. In others it increased slowly. The degree of plethora ultimately attained likewise varied much. An amount of blood which in one animal resulted in a rise of fifty points in the hemoglobin per cent, produced in another individual of the same size a rise of only thirty points. For this reason some of the animals were transfused more often than others. Even so it was often found impossible to force up the hemoglobin per cent in these refractory individuals to a height easily reached in other animals. The shortest period of transfusion was 9 days, during which the animal was given seven transfusions; the longest, 20 days with nineteen transfusions. As a rule, the hemoglobin per cent rose from a normal of 80 to 90 per cent to 140 to 150 per cent, at which point it remained fairly constant despite the continued introduction of blood.

No notable change in the percentage of reticulated cells occurred until after several transfusions. Then, as plethora became well defined, the number of reticulated cells in the circulating blood began to diminish (Text-figs. 1 and 2). This decrease in number was sometimes rapid and soon became marked. Often the reticulated cells practically disappeared. Counts below 1 in 10,000 were frequent (Text-fig. 1), and sometimes a search through a whole slide would reveal none. The charts of Text-figs. 1 and 2 will illustrate the change that occurred in eight of the sixteen recipients. In five others, the drop in reticulated cells was less marked, though definite. They decreased to about 1 in 1,000. The degree of plethora was fully as great in these animals as in the afore mentioned ones, and no reason has been found for the differences observed.

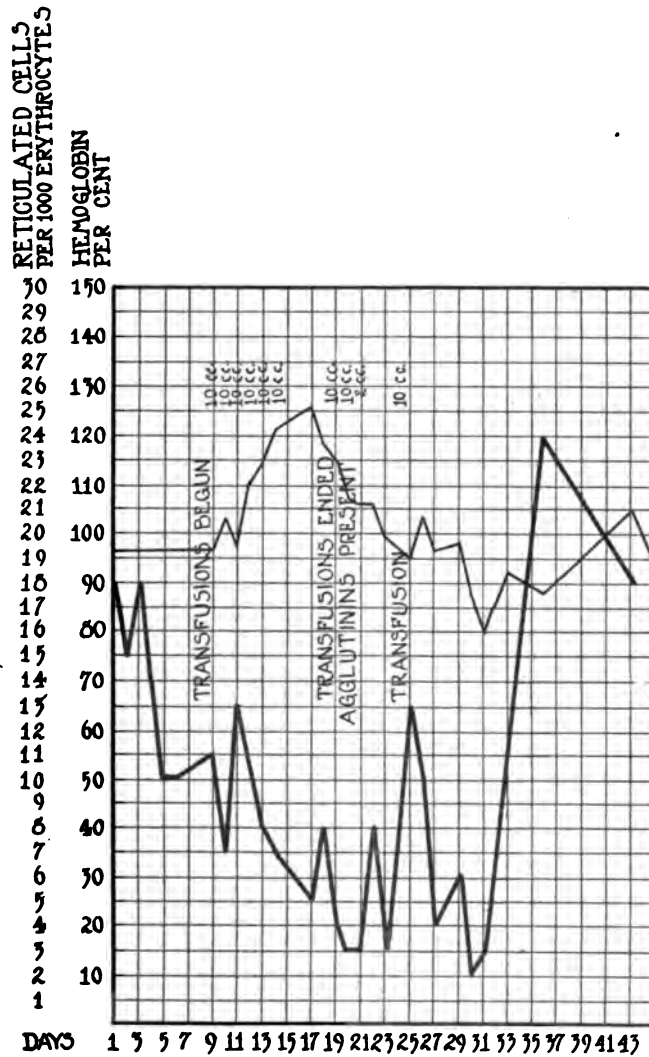
Anomalous results were obtained in three rabbits. One showed only a slight drop in the reticulated red cells (Text-fig. 3). In the remaining two, these cells failed to decrease at all. One had a purulent pneumonia, with some anemia, despite the transfusions. The other developed an increased number of reticulated cells for which no cause was ever found.



TEXT-FIG. 1.

TEXT-FIG. 2.

— Reticulated cells.
 — Hemoglobin.



It is evident from these results that a diminution in the number of reticulated cells in the circulation follows almost constantly the production of an artificial plethora. A possible influence of sodium citrate in causing this change has been ruled out by observations in five rabbits which received daily injections of sodium citrate alone, in some cases twice as much being given as in the rabbits made plethoric. The number of reticulated cells did not decrease in these animals.

Examination of the Bone Marrow.

The bone marrow from five of the eight rabbits in which the reticulated cells dropped markedly was examined for its content of these cells. The following procedure was employed.

Red marrow from the upper and lower ends of the femur was taken into a mixture of equal parts of Locke's solution containing $\frac{1}{2}$ per cent of gelatin and an isotonic watery solution of sodium citrate. In this mixture clotting does not occur, and even the most delicate cells are well preserved. The gross appearance of the marrow was normal except for a congestion such as was present in all the other organs. A small portion of it was teased on a slide in a drop of the Locke's-citrate mixture and a count made at once from the preparation. Then, with a view to washing out the cells of the marrow, the needle of a syringe was thrust into it here and there, and gelatin-Locke's solution injected under considerable pressure. In this way a large part of the substance of the marrow was washed out. The washings were then centrifuged and counts made on the sedimented cells according to the technique used for the peripheral blood. To obtain a figure for purposes of comparison, an average was taken from the counts on the teased specimen and the washings.

The average number of reticulated cells in the bone marrow of the five plethoric animals was 14 per 1,000 red cells, the highest number 22 and the lowest 5 per 1,000. In striking contrast are the figures obtained in the examination of five normal rabbits. These showed an average of 320 per 1,000 red cells, the highest 540 and the lowest 160 per 1,000.

Anemia Following Plethora.

In certain of the plethoric rabbits there occurred, after many transfusions, a sudden marked drop in hemoglobin. In one instance (Text-fig. 4), despite daily transfusions of 10 cc. of blood, the hemo-

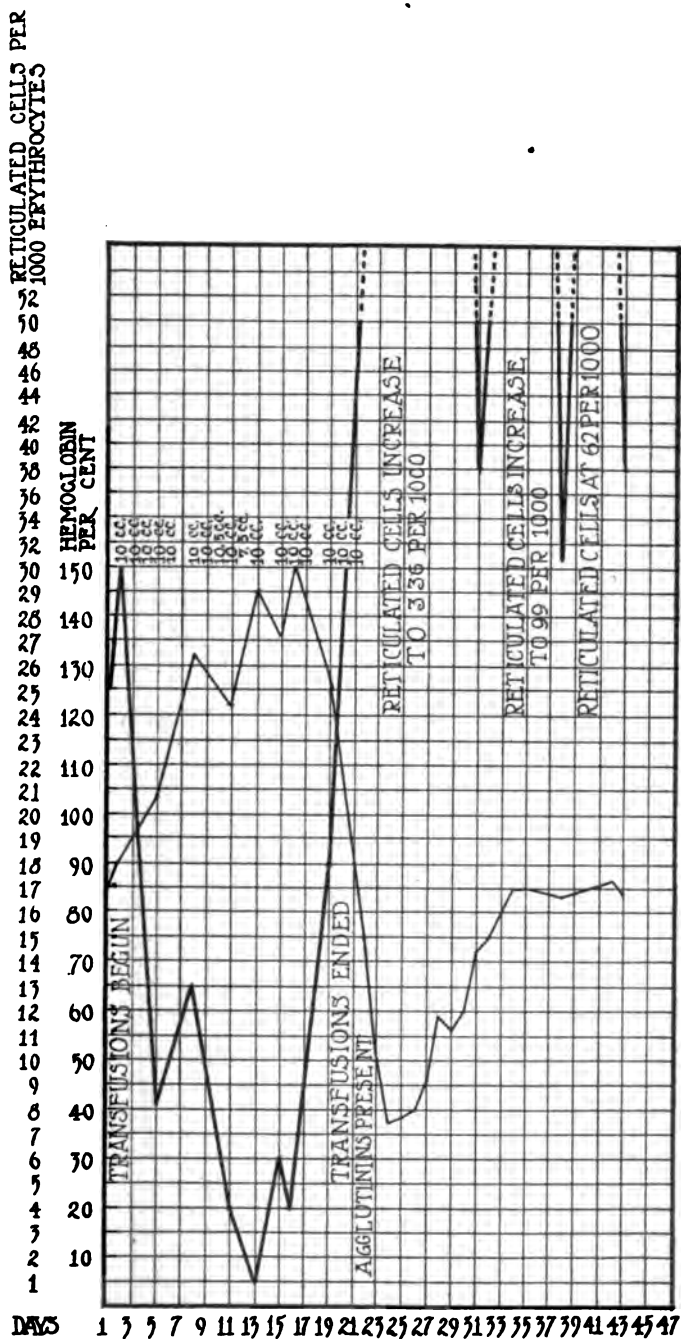
globin fell in 6 days from 150 per cent to 75 per cent. Transfusions were discontinued in such animals, and there rapidly ensued a severe anemia. This was observed in three rabbits. Charts of two of them are shown in Text-figs. 4 and 5. The cause of the rapid transition from plethora to anemia has not yet been determined, but it is significant that isoagglutinins for the donors' blood developed in all three rabbits at about the time when the hemoglobin first began to fall. The simplest explanation would seem to be that, following a number of transfusions, a lytic process develops against the strange cells, and they are rapidly destroyed. This results not merely in a return of the hemoglobin to normal but in an anemia, because of the peculiar condition of affairs indicated by the drop in reticulated red cells during the plethora. During it the bone marrow activity sinks far below normal, whereas blood destruction is maintained at the normal rate at least. Thus, little by little, as the animals' own cells are destroyed and not replaced, the bulk of the circulating blood comes to be strange blood liable to destruction when the lytic principle develops. The extent to which the blood cells proper to the recipient have been destroyed and replaced by alien cells is suddenly revealed through the destruction of this alien blood.

The anomalous behavior of one rabbit, whose chart is shown in Text-fig. 3, strongly supports this explanation of the phenomenon. In this animal there came at length a drop in the hemoglobin, but instead of progressing to an anemia, as in the three rabbits described above, it sank to a point only slightly below normal. The failure of anemia to develop here would seem to be due to the fact that during the plethora, blood production had been maintained at almost its normal rate as indicated by the lack of any but a slight diminution in the number of circulating reticulated cells.

The recovery from the anemia in the three rabbits just mentioned was exceedingly rapid. A similar rapid recovery was noted by Muir and M'Nee⁹ and Itami,¹⁰ following experimental hemolytic anemias.

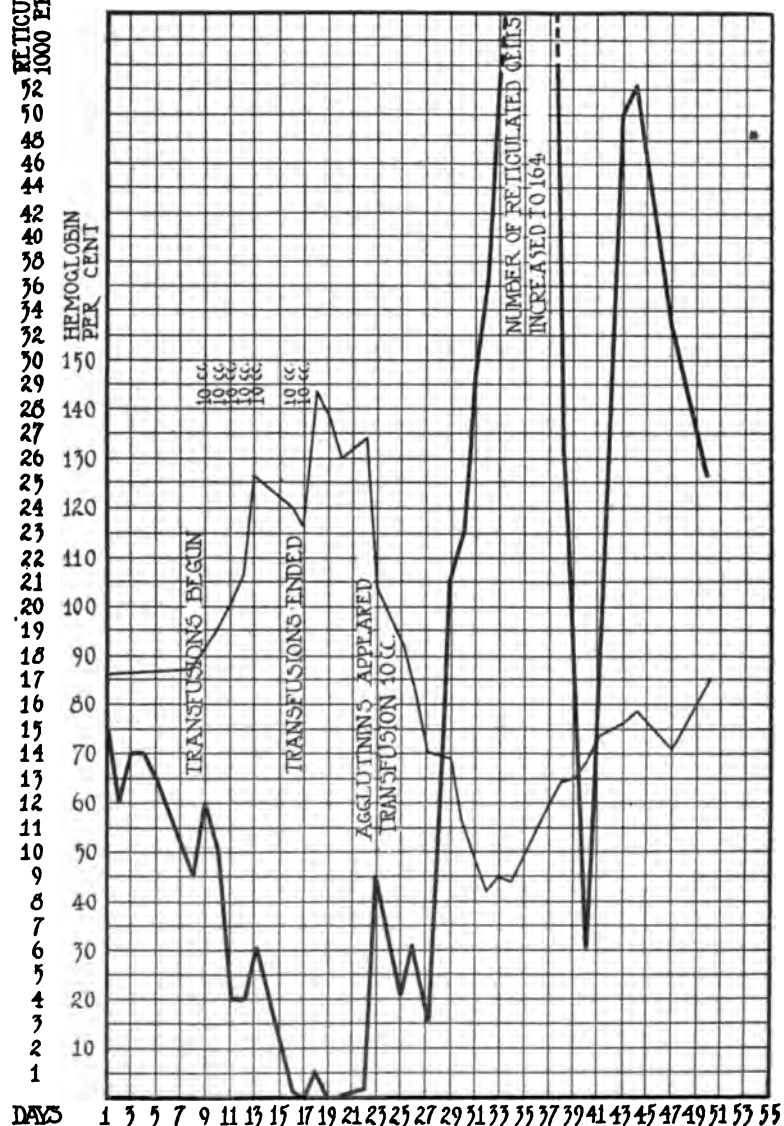
⁹ Muir, R., and M'Nee, J. W., *J. Path. and Bacteriol.*, 1911-12, xvi, 410.

¹⁰ Itami, *Arch. exp. Path. u. Pharm.*, 1910, lxii, 104.



TEXT-FIG. 4.

RETICULATED CELLS PER
1000 ERYTHROCYTES



TEXT-FIG. 5.

Increased Bone Marrow Activity During Subsidence of the Plethora.

The beginning hemoglobin drop in those rabbits which developed an anemia during or after transfusion was marked in each case by a prompt rise in the number of reticulated cells. This increase was rapid, and in two cases the number had risen above normal by the time the hemoglobin had descended to its original level (Text-fig. 4). Then, as the hemoglobin fell further and anemia developed, the reticulated cells continued to increase with great rapidity. The largest number was observed during the early period of regeneration. One rabbit showed a maximum of 594 per 1,000 at this time. They gradually diminished as the animal recovered from the anemia but were still increased when the hemoglobin reached normal again and remained fairly numerous for some time.

Attention is called to the fact that the number of reticulated cells began to increase soon after the hemoglobin started to fall from the plethora level and long before it had reached normal. A further study of this phenomenon was considered worth while. It seemed not unlikely that stimulation of the bone marrow at this time might be brought about by the greatly increased quantity of destroyed blood present. Accordingly an attempt was made to determine this possibility by injecting rabbits intravenously with laked blood. The rabbits were first rendered anemic¹¹ by bleeding, and the injections of blood were made at different stages of recovery. Some of the rabbits were treated when very anemic; others had practically reached normal before treatment was begun. In order to simulate as nearly as possible conditions of blood destruction occurring *in vivo*, the laked blood was injected at 1 hour or 2 hour intervals for periods of 1 to 3 days. Relatively large quantities were given without apparent ill effect. At no time during the course of the experiment was any increase in the number of reticulated cells noted, nor was other evidence obtained of increased bone marrow activity.

It was then found that simple blood removal from a plethoric animal by bleeding was sufficient to cause a marked bone marrow

¹¹ The reason for producing a preliminary anemia in these rabbits is that the experiment was performed originally in an attempt to explain on experimental grounds the cause of the remission in pernicious anemia.

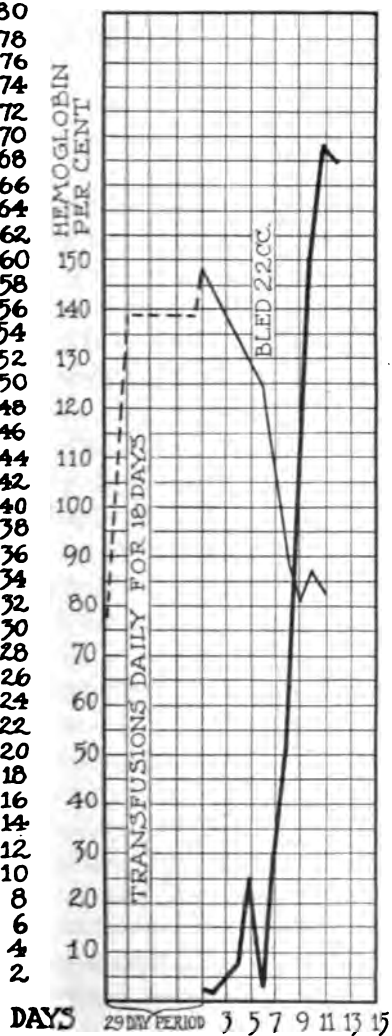
stimulation. Rabbits were made plethoric in the usual way and kept in this condition until the reticulated cells were much diminished. They were then bled a quantity calculated to bring their hemoglobin per cent back to almost normal. An immediate rise in the number of reticulated cells resulted. The normal number, it will be recalled, is 5 to 20 per 1,000 red cells. In one animal treated as above described, they increased to 69 per 1,000 (Text-fig. 6); in another to 80. A third rabbit showed even a greater increase, 108 per 1,000 (Text-fig. 7), but unfortunately the hemoglobin in this case fell after bleeding to slightly below normal. With a fourth animal, the increase was definite, but less marked. In Text-fig. 6 it will be noted that the fall in hemoglobin following bleeding extended over a period of several days, and that the reticulated cells had increased markedly some time before the hemoglobin reached its normal level. Text-fig. 7 shows an instance in which the drop in hemoglobin as a result of bleeding was later followed by a rise above normal. This secondary rise in hemoglobin and the high reticulated cell count were maintained for some days.

Although these experiments are not sufficiently complete to permit one to draw definite conclusions, yet the results would suggest that the increased bone marrow activity accompanying the initial drop of hemoglobin in the plethoric rabbits is due to some functional disturbance of the circulation, resulting from the rapid removal of the plethora. It seems not improbable that this may be a temporary relative oxygen deficiency explainable on the basis that during the period of plethora the organism had in some way adapted itself to a blood of greater oxygen-carrying power. Certainly the blood loss may be thought of as having resulted in a relative anemia.

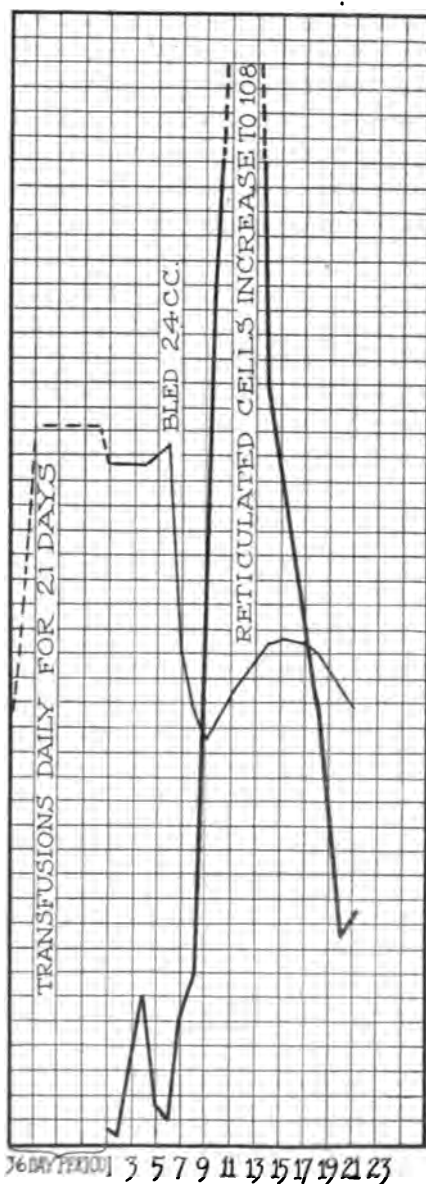
Color Index.

Observations on the color index in those plethoric rabbits developing anemia revealed striking changes. The normal color index for rabbits is 0.65 to 0.75. During regeneration it was greatly increased, usually to 1.0 or even to 1.08. This finding is of interest particularly in its relation to the accompanying greatly increased percentage of reticulated cells in the blood. It would indicate that reticulation is

RETICULATED CELLS
PER 1000 ERYTHROCYTES



TEXT-FIG. 6.



TEXT-FIG. 7.

not necessarily associated with a deficiency in the hemoglobin content of the cells and that this association in the blood of secondary anemia is largely fortuitous. In two of the present instances, the reticulated cells numbered 34 per cent and 60 per cent respectively of the total number of red cells, yet the color index was increased 50 per cent over the normal. Even allowing for the somewhat increased size of the reticulated cells, it does not seem possible that they contained less hemoglobin per unit of cell substance than the accompanying more normal red cells. In fresh films they were observed to be notably well colored.

Transfused Reticulated Cells.

In the work as thus far discussed, a possible source of error has not been considered; namely, that of the reticulated cells introduced with the transfused blood. The number of these may, and indeed sometimes must be, considerable, because the bone marrow of the animals furnishing the blood has been stimulated by repeated bleeding. After many transfusions one might expect the recipients to show high counts of reticulated cells. As a matter of fact the reverse is the case. What becomes of the reticulated cells introduced, whether they perhaps mature into non-reticulated corpuscles or instead are destroyed, remains to be determined. As bearing on the point, it should be mentioned that the microcytes of secondary anemia due to hemorrhage are largely derived from fragmentation of reticulated cells, and that in plethoric animals the fragmentation of red blood cells is much increased.⁷

No observations were made on the effect of plethora on the number of white cells or platelets.

Clinical Bearing.

The effect of experimental plethora on the bone marrow of rabbits has a direct bearing on certain unfavorable results which may occur after transfusions in human beings. Clinicians have observed that some cases of pernicious anemia receiving transfusion show no stimulation, but instead unmistakable signs of bone marrow depression.

Vogel and McCurdy⁶ were the first to report a systematic study of the reticulated cells in pernicious anemia patients with transfusion. In several of their cases, a decided drop in the per cent of reticulated cells occurred following transfusion. They attributed this to the diluting effect of the newly introduced blood, which had a much lower content of reticulated cells. These patients did badly.

At the Massachusetts General Hospital, Minot and Lee¹² have recently observed the effect of transfusion on the per cent of reticulated cells in a number of cases of pernicious anemia. In certain of their patients, a very marked diminution in the reticulated cells occurred following transfusion. This was accompanied by a leukopenia, a reduction in the number of platelets, and in some cases purpura as well, all of which went to form the picture of general bone marrow depression. As was to be expected, there was no increase in the red corpuscles after transfusion in these patients, other than that referable to the alien blood introduced. In their cases, the amount of blood transfused did not exceed 600 cc. and in one case it was only 300 cc. No such effects as those described above were observed after transfusions of less than 300 cc.

From the results described in the present paper, one may draw tentative conclusions as regards such instances. In pernicious, as in any form of anemia, oxygen deficiency resulting from blood loss doubtless constitutes the stimulus for increased blood production. It is conceivable that in severe conditions, a stage is reached where the bone marrow becomes so exhausted that there is danger of its failure to respond any longer to this stimulus; in other words, the stimulus of oxygen deficiency has grown relatively less effective and may at any time become insufficient. The introduction of a large quantity of blood into the circulation has inevitably the effect of reducing oxygen lack. The sudden lowering of stimulus thus brought about may result in a diminished activity of the bone marrow. The inference is clear that in pernicious anemia with a sluggish bone marrow as shown by the count of reticulated cells, small transfusions are preferable to large ones.

¹² Dr. Minot and Dr. Lee have very kindly allowed me to use these data, which they have not yet published.

SUMMARY.

With the purpose of determining whether a diminished activity of the bone marrow could be brought about experimentally, plethora was produced in rabbits by means of repeated small transfusions of blood. Counts of the number of reticulated red cells in the circulating blood were made during the course of the experiments as an index to changes in the activity of the bone marrow.

With the development of plethora, the number of reticulated cells in the blood decreased. In the majority of the plethoric animals, this diminution was extreme, and in some instances, reticulated cells practically disappeared from the blood. A comparison of the red bone marrow of these animals with that of normal controls revealed a marked reduction in the content of reticulated cells.

After a number of transfusions, there occurred in some of the plethoric rabbits a sudden and marked drop in hemoglobin. The hemoglobin continued to fall until a severe grade of anemia was reached. This was followed by an extremely rapid regeneration accompanied by a striking rise in color index. During regeneration, the reticulated cells were enormously increased in number.

Taken together, these facts show that the bone marrow is markedly influenced by plethora. The diminished number of reticulated cells observed, both in the circulating blood and in the marrow, would make it appear that a decided decrease in blood production occurs. The reduction in the number of these cells cannot be due to changes in the constitution of the red cells put out by the bone marrow, as a result of an increased quantity of hemoglobin in the body, because during regeneration from the above mentioned anemia, when the color index was very high, reticulated cells were still present in large numbers. That the activity of the bone marrow does actually diminish during plethora is further evidenced by the occurrence of the anemia. The most reasonable explanation of this phenomenon is that the recipient develops an immunity against the blood of the donors, which results in the destruction of the strange cells that are in circulation. In keeping with this conception is the appearance of isoagglutinins for the donors' red cells in the blood of the recipient, at about the time of the beginning fall in hemoglobin. The occurrence of anemia as a result of the destruction of the alien blood only would seem to be

due to the circumstance that, during the period of plethora, blood production is greatly diminished; as a consequence, the blood cells proper to the recipient are gradually reduced in number and replaced by alien cells until the latter come to constitute the bulk of the animal's blood.

In those rabbits developing anemia, the initial drop of hemoglobin from the plethoric level to the normal was constantly accompanied by a marked rise in the number of reticulated cells. This brought up a subsidiary problem for study. With the idea that the stimulation of the bone marrow might be due to the presence of an increased quantity of broken down blood, rabbits were injected intravenously with large amounts of laked blood cells. The procedure had no evident effect on the blood picture. It was then found that simple blood removal from a plethoric animal which brought back the hemoglobin to the normal level, or even to a point somewhat above, sufficed to cause a marked increase in the number of reticulated cells. Although these findings are not conclusive, they suggest an explanation for the increased bone marrow activity accompanying the initial drop of hemoglobin in the plethoric rabbits; namely, that the organism had in some way adapted itself during the period of plethora to the presence of a greater amount of blood and that the result of blood loss in such an organism was a relative but not absolute anemia.

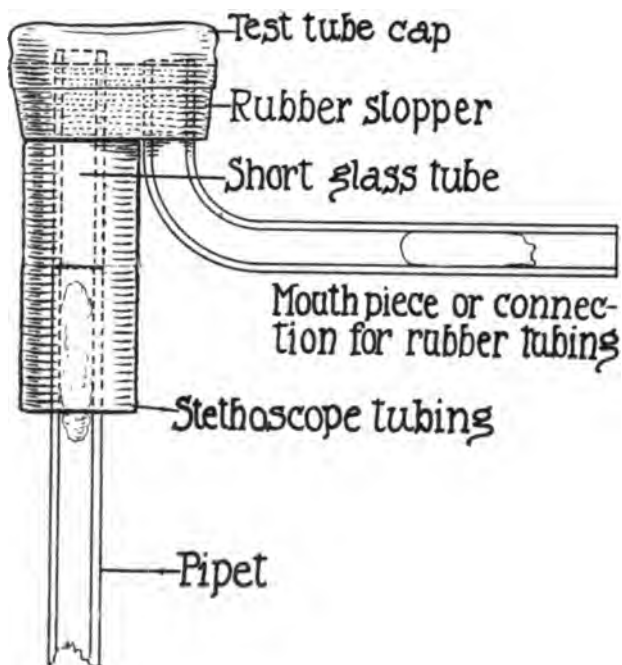
The finding that the activity of the bone marrow can be depressed by the introduction of a large quantity of blood into the circulation accounts for the diminished bone marrow activity which sometimes occurs after transfusion in pernicious anemia. In such cases there is a marked drop in the number of reticulated cells and other evidence of bone marrow depression; the patient shows no benefit from transfusion or may grow rapidly worse. The cause of this depression is best explained on the basis that in severe instances of the disease where exhaustion of the bone marrow is imminent, the stimulus of the anemia is only just sufficient to keep the marrow functioning. A sudden lowering of this stimulus is brought about by the introduction of a large quantity of blood into the circulation, and the result is a fall in the activity of the bone marrow. It follows from this that in pernicious anemia with a feebly reacting bone marrow as indicated by the number of reticulated red cells, small transfusions are preferable to large ones.

A SAFETY CAP FOR GRADUATED PIPETS.

By FREDERICK L. GATES, M.D.

(From the Department of Pathology and Bacteriology, The Rockefeller Institute for Medical Research.)

A certain number of laboratory infections are traceable to the handling of pathogenic micro-organisms in pipets which are filled by suction with the mouth and emptied gradually by the admission of air under the ball of the finger. Such accidents may occur either from



Safety cap for graduated pipets.

infection of the end of the pipet by a contaminated finger or from sucking organisms into the mouth, an occurrence against which cotton plugs are not a sure protection.

Rubber bulbs and other simple vacuum and pressure devices are not sensitive enough for use with graduated pipets when accurate control of the meniscus is desired. Even when carefully handled, the elasticity of the contained air is a disturbing factor.

With semifluid cultures also, such as are used in the cultivation of spirochetes, the negative pressure of a collapsed rubber bulb is not sufficient to cause the medium to be forced into a capillary pipet.

The safety cap illustrated herewith obviates the danger mentioned, and yet gives accurate control of the delivery of liquid from the pipet. It consists of a section of two-hole rubber stopper (No. 0 or 1), 1 cm. thick, over which a flat rubber test tube cap¹ is slipped. It may be cemented in place, although that is not necessary. An L of glass tubing forms an easily sterilized mouthpiece, which may be further safeguarded by a cotton plug.

Two cotton plugs and the space in the cap are almost a sure protection against the aspiration of liquid. Connection with the pipet is made by a stub of straight glass tube, which projects into the cavity of the cap and carries a short length of heavy rubber stethoscope tubing. This is more elastic than a rubber stopper, and admits various sizes of pipets.

To give a stiff resistance to pressure from above, the pipet should be pushed into contact with the short glass tube. The finger, pressing the smooth, thin rubber cap down on the end of this tube, controls the delivery of liquid with perfect sensitiveness. Fractions of a division on the pipet may be measured, and the column of liquid is obedient at any speed of delivery.

If from strong suction in filling the pipet the cap tends to collapse over the glass tubes it may be held away by pressure of the forefinger against its rounded edge.

An additional advantage of the safety cap is that the pipet is held at an angle with the line of vision, so that the meniscus is easily visible as it rises in the bore. When several pipets are in use at one time, it is convenient to fit each of them with a cap.

1. Eimer and Amend: Catalogue C, Fig. 6026, $\frac{1}{4}$ inch.

A VALVE TO REGULATE THE DELIVERY OF AIR AND ' ETHER VAPOR IN ANY PROPORTION.

By FREDERICK L. GATES, M.D.

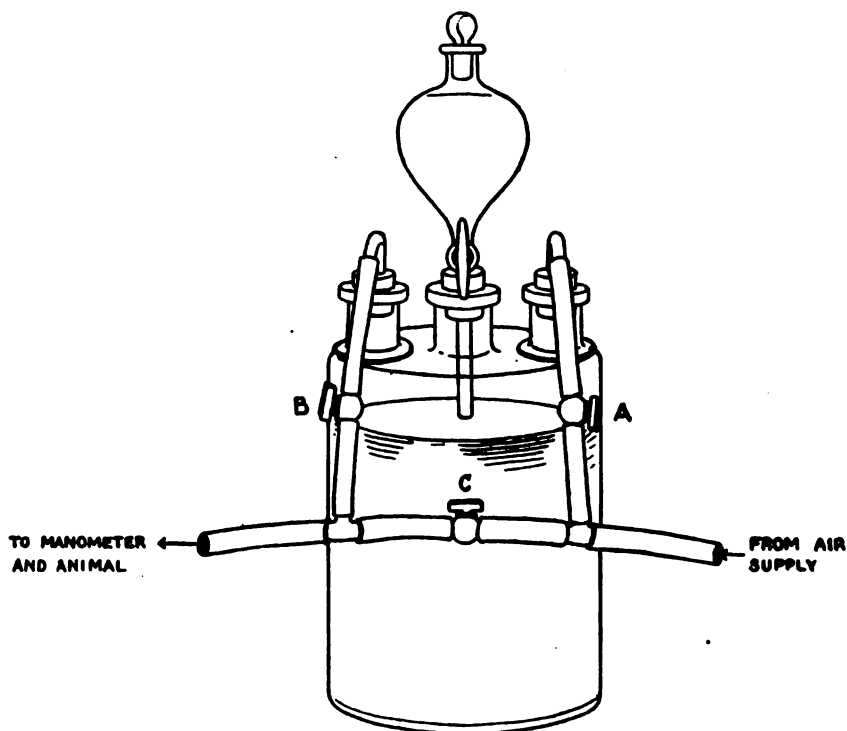
(From the Department of Physiology and Pharmacology of The Rockefeller Institute for Medical Research.)

(Received for publication, March 5, 1917.)

Since the introduction of the Meltzer-Auer method of intratracheal insufflation for artificial respiration and anesthesia in 1909, this method of delivering air and ether vapor under positive pressure at the bifurcation of the trachea has been widely adopted in laboratories of research and in the surgical clinic. The close regulation of air pressure and ether vapor tension, the constancy of delivery independent of the respiratory movements of the anesthetized subject, and above all, the safety and surety of the method in supporting life in spite of respiratory failure, combined with smoothness of anesthesia and freedom from danger of aspiration pneumonia, make it the most satisfactory method for human as well as laboratory surgery.

An extensive literature has grown up in this country and abroad which by its character attests the success of the method in principle and practice. Most of the authors agree on the correctness of the principles involved; most of them have some contribution to make in the way of new apparatus for the simplification and perfection of the technique or the mechanics of the method. Many types of respiration machines have been described, their variety proving the wide flexibility of the method and its adaptability to individual preference and need.

Dr. Meltzer has always insisted upon the utmost simplicity in apparatus, and in his laboratory a consistent attempt has been made to reduce the mechanics of intratracheal insufflation to the lowest terms consonant with efficiency. In this way mistakes and complications may be avoided and the mind of the operator freed from constant supervision of the respiratory apparatus and the anesthesia.



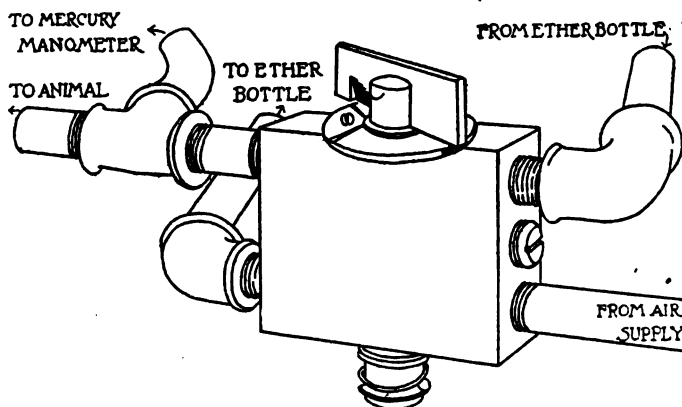
TEXT-FIG. 1. Woulfe bottle with the usual arrangement of tubing and stop-cocks for air and ether vapor control. One-fourth the actual size.

Method of Obtaining Ether Vapor.

The simplest and most reliable method of obtaining ether vapor for insufflation anesthesia is the passage of the part of the air stream over the surface of ether in a Woulfe bottle (Text-fig. 1). The level of the ether is maintained by an auxiliary supply in a separating funnel. This is the method long in use in Dr. Meltzer's laboratory and has been adopted, with modifications, in several of the respiration machines intended for use in the clinic. If the ether surface is ample, it is not necessary to bubble air through the ether, or to warm the ether to promote evaporation, both of which are questionable procedures not unattended with danger.

Heretofore the diversion of a portion of the air stream over the

ether surface has been accomplished by means of stop-cocks in the tubing or some equivalent arrangement (Text-fig. 1). Three cocks are necessary for proper regulation of the ether supply. A and B control the air current in the ether circuit, while C regulates the passage of air unmixed with ether vapor. This arrangement has the disadvantage that any desired change in the proportion of ether vapor requires the manipulation of all three cocks to maintain the stream at its former volume and pressure, and even then it is practically impossible to determine the proportion of vapor-laden air obtained. It may be emphasized further that it is essential to have cocks A and B open to the same degree, lest an excess of ether vapor



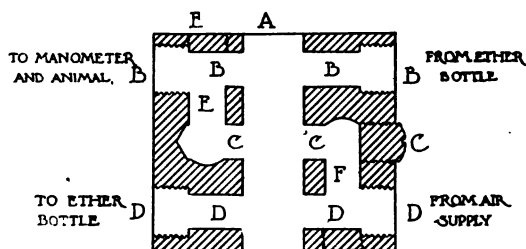
TEXT-FIG. 2. Constant volume valve, with fittings for the ether bottle and manometer. One-half the actual size.

be drawn into the tubing by the passage of air through C, or enter the system during the interruption of the air current if only one of the passages to the Woulfe bottle is subject to regulation.

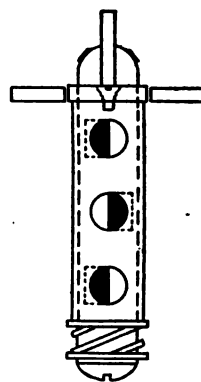
Description of the Valve.

The constant volume valve here described (Text-fig. 2) is in essence a convenient means of regulating all three stop-cocks synchronously, so that as the air stream over the ether is increased, the stream through C is reduced in exact proportion, and the sum of the volumes passing through the two limbs of the divided circuit remains constant. It consists of three channels corresponding to A, B, and C in Text-

fig. 1, but all contained in a single block, and all regulated synchronously by a single cock key through which the three channels are drilled. In the block (Text-fig. 3), D and B are the passages to and from the ether bottle, and C is the direct air passage in connection with D, F and E, B. As the passages B and D are closed by turning the cock key A, passage C must open in exact proportion so that the sum of the openings D (ether) and C (air) shall always equal a single opening of the same cross-section. This is accomplished by drilling the key so that the left edge of the holes corresponding to B and D are in line with the right edge of the hole corresponding to



TEXT-FIG. 3.



TEXT-FIG. 4.

TEXT-FIG. 3. Longitudinal median section of the brass block, drilled and plugged. One-half the actual size.

TEXT-FIG. 4. Cross-section of the block near the key showing the arrangement of the square ports in the key in relation to the passages for air and ether vapor. One-half the actual size.

C. Text-fig. 4 shows a cross-section of the block close to the key, which is set for half ether, half air. Moreover, the holes in the key must be square, cutting off segments with the circular holes in the block, the sum of which equals the cross-section of a single channel.

The dial plate over which the handle of the cock key turns is calibrated to show the proportion of the air stream which is sent over the ether in the Woulfe bottle. It should be pointed out that this is not an absolute index of the tension of ether vapor obtained, as that is further dependent upon the rate of evaporation of the ether

and the rate of flow of the air stream. We have not found it necessary or desirable in ordinary anesthetics to determine the vapor tension of the ether with accuracy. Many complicating factors come into account which make a record of ether tension, either in millimeters of mercury or in percentage of ether vapor, merely an abstract number, useful only for the reestablishment of similar conditions at some later time if desired. This can be done with sufficient accuracy for ordinary purposes by resetting the key handle on the dial scale to the point previously determined. It should be emphasized that the condition of the patient is always the criterion of the amount of ether to be given. The anesthesia is to be regulated by observation of the patient, not by the ether vapor tension on a scale. Even in the laboratory, where there is less at stake than in the clinic, we find that different animals of the same species require different amounts of ether to produce safe anesthesia under apparently similar conditions.

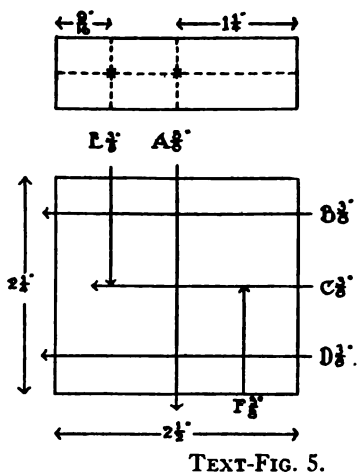
The valve described above has proved a simple and reliable means of regulating the ether vapor tension. Lately we have been employing this method of ether regulation for anesthesia in small animals, cats, rabbits, guinea pigs, rats, and mice, directing an ether-laden air stream to an ordinary cone, as used in the open drop method. The great advantages of intratracheal insufflation are not obtained, but the superiority over the open drop method of an unvarying supply of dry ether vapor, subject to close and instant regulation, is apparent. For one thing, the time of an assistant is saved.

Directions for Constructing the Valve.

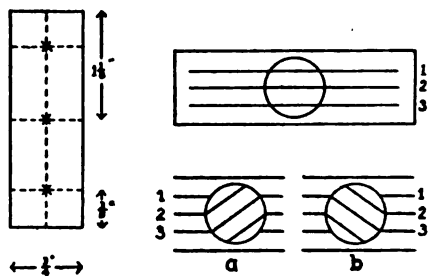
Since the valve can easily be turned out by a good technician or mechanic, working directions for its construction are appended.

The block is of brass, $2\frac{1}{2}$ by $2\frac{1}{4}$ by $\frac{3}{4}$ inches. Text-fig. 5 shows the centers and directions for drilling the channels. Hole A, $\frac{5}{8}$ inch in diameter, is for the key and should be drilled first, since it must be straight and true. Then B, C, and D, $\frac{3}{8}$ inch in diameter, are drilled in turn, B and D through the block, C to within $\frac{3}{8}$ inch of the opposite end. E and F, $\frac{3}{8}$ inch in diameter, join B to C at one end of the block and D to C at the other. B and D are drilled out at each end and tapped for $\frac{1}{4}$ inch pipe fittings. After the proper holes are

drilled through the key, C, E, and F are tapped out and plugs screwed in as far as F, B, and D respectively. The end of the plug in C may be cut as a screw head and used to regulate the size of F if the resistance of the passages to the ether bottle is found to be higher than that of the direct air passage C. The other plugs may be cut off flush with the block. A median longitudinal section of the block as drilled, tapped, and plugged is shown in Text-fig. 3.



TEXT-FIG. 5.



TEXT-FIG. 6.

TEXT-FIG. 5. Dimension drawing of the block as laid out for drilling. One-half the actual size.

TEXT-FIG. 6. Bottom of the block with lines for setting the key. a, position of the key when C is drilled through it. b, position of the key when B and D are drilled. One-half the actual size.

The valve key, to fit properly, should be turned down to $\frac{5}{8}$ inch from larger stock and ground in with a very little emery and oil. A heavy grease used for lubrication will obviate any slight leak. The key is $3\frac{1}{4}$ inches long. The valve handle, of strip brass 2 by $\frac{3}{4}$ by $\frac{1}{8}$ inches, is riveted in a slot in the key, the other end of which projects $\frac{3}{8}$ inch beyond the bottom of the block and is fitted with washers and a spiral spring of moderate tension held in place by a screw or cotter-pin.

To drill the key properly, set the handle parallel with the long way of the block and draw three lines on the bottom of the block and key

as shown in Text-fig. 6. Line 2 is median, and lines 1 and 3 are parallel and $\frac{3}{8}$ inch from 2. Turn the key until 2 on the key intersects 3 and 1 on the block (Text-fig. 6a). Drill C through the key. Then turn the key back until 2 on the key intersects 1 and 3 on the block (Text-fig. 6b), or, better, until the exact point is reached at which air will no longer pass through C. Then drill B and D through the key. This is the position of the key for full ether. Turning the key back to the first position, it will be discovered that C (air) is fully open and B and D are just closed. Intermediate positions of the key handle will give various mixtures of ether vapor and air. Square up the holes in the key with a small square file. A dial plate made of a large brass washer (Text-fig. 2) may be attached to the block by machine screws and calibrated mathematically or by test to show the proportion of ether-laden air in tenths of the total volume. As described, the valve is tapped for $\frac{1}{4}$ inch pipe fittings as follows:

D, short pipe for tubing from the source of the air supply.

B and D', close nipple, elbow, short pipe for tubing to the ether bottle.

B', short nipple, tee, short pipes for manometer and for tubing to the animal.

The test of the valve's efficiency is that under working conditions it shall maintain a constant pressure in the mercury manometer when the valve handle is turned slowly between full ether and full air. If there is a rise in the pressure in the direction of full air, the resistance of the air circuit may be raised to that of the ether circuit by screwing in the plug C until it begins to cut off passage F.

SUMMARY.

A valve is described for the control of ether vapor for anesthesia which regulates the mixture of ether vapor from a Woulfe bottle with air in any proportion, without changing the volume or the pressure at which the mixture is delivered. The regulation of the air stream both to and from the ether bottle controls the mixture accurately and is an essential feature of the valve. Except for experimental purposes, it is not considered profitable to determine on an arbitrary scale the tension of the ether vapor obtained, because the depth of the anesthesia should always be judged by the condition of the subject.

The valve was devised for the Meltzer-Auer method of intratracheal insufflation, but it is adapted for use wherever a constant mixture of air and ether vapor is desired. Several respiration machines for supplying and interrupting the air stream, using this constant volume valve for the regulation of the ether supply, have been in use in Dr. Meltzer's laboratory for periods up to 2 years, and the simplicity and efficiency of the valve have been thoroughly tested. A description of the complete machine will be published later.

HISTORY AND ANALYSIS OF THE METHODS OF RESUSCITATION.

WITH A DESCRIPTION AND A DISCUSSION OF THE AUTHOR'S PHARYN-
GEAL INSUFFLATION APPARATUS FOR ARTIFICIAL
RESPIRATION IN MAN.

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I. Historical Review.

It is quite probable that from time immemorial men tried to revive the apparently dead, and that for accomplishing this end employed all sorts of procedures; but our actual knowledge of these procedures does not reach very far back. The verifiable history of resuscitation is only about 150 years old. The society which was organized for this purpose in Amsterdam, 1767, seems to have made the first attempt to lay down definite rules for resuscitating the apparently dead; at least it was the first attempt which left a record. The rules were embodied in a little book which was translated in 1773 by a Dr. Cogan into English. That booklet stimulated the public spirited Dr. William Hawes to establish in London an organization under the name of The Humane Society, which had in view an object similar to that of the Amsterdam Society, and which is still in existence under the name of The Royal Humane Society. At the time when The Humane Society was established, England was fortunate to possess many illustrious medical men, some of whom took an active interest in the development of the subject of resuscitation. I need only mention such medical stars as John Hunter, who was a director of that society; his brother William Hunter, Cullen, Monro secundus, and Fothergill. It should be mentioned that de Haën published in 1772 results of an "experimental inquiry into the phenomenon of drowning and resuscitation as seen in dogs." De Haën was then professor of medicine in Vienna. But he came from Holland, was a

pupil of Boerhaave, and it was probably the apparently dead from drowning in Dutch waters, and not in the waters of the Danube River, which made him look experimentally for developing methods of resuscitation. It was again then Holland which gave the stimulus to experimental studies of life-saving methods. At any rate, it was in the eighth decade of the eighteenth century that procedures of resuscitation assumed a scientific aspect, began to be systematized, became a medical subject, and found a permanent place in the medical literature.

The latter fact is in a great measure due to the activities of The Royal Humane Society, which from the start kept records of all cases of attempted resuscitation which came to its knowledge. At various intervals it formulated and published rules, in conformity with the views which prevailed at the time, and stimulated individuals to and formed committees for special investigations with the object of discovering reliable methods to be employed. In several interesting papers, Arthur Keith¹ made us acquainted with some historical data and with the details contained in the records of the society. At the time the mentioned societies were founded the subjects to be resuscitated were chiefly those apparently dead from drowning; hence, the special interest which Holland and England took in the matter. However, the progress made since this time in industry, science, and medicine created new victims in its train. The introduction of gas for the purpose of lighting and heating, the extensive development of mining, of the knowledge and the technical application of electricity, the discovery of ether, chloroform, and other anesthetics, the growth of the domains of surgery, the discovery and popularization of narcotic drugs; all these blessings multiplied greatly the unfortunate opportunities for the necessity of employing methods of resuscitation.

Did the methods improve with the growth in the necessities of their application? I shall try to answer by giving a brief outline of the history of resuscitation which may be justifiably divided into two periods, the year 1856 being the dividing line. In the last 60 years resuscitation consisted and still consists practically exclusively in the application of artificial respiration in one form or another. During the first period, however, besides artificial respiration many other

procedures were employed which may be collectively described as stimulations. The earliest procedures consisted in the application of heat to the body, of various stimulants to the skin, the nose, etc., in blood-letting, in the production of vomiting "by the French method." An interesting procedure was that of "fumigation;" it consisted in filling the large bowel with tobacco smoke. "For nearly 40 years great importance has been attached to this curious procedure. The record of case after case bears witness to the success ascribed to this method." The disappearance of fumigation from the list of permissible means of resuscitation is characteristic for the "ups and downs" in the history of therapeutics in general. Benjamin Brody found in three experiments that the infusion of 4 ounces of tobacco, administered per rectum, would kill a dog, and one ounce a cat. The Royal Humane Society became alarmed and relegated fumigation to the list of forbidden rules (Keith). The use of artificial respiration as a method of resuscitation had rather a variable fate during this period. Aside from the barrel method (rolling those apparently dead from drowning on a barrel), artificial respiration was performed by inflation of the lung from mouth to mouth while the nose of the victim was kept closed, a method which seemed to have been long in practice in reviving the still-born and which, in this domain, is occasionally still in use. However, about 1776, the bellows method came to the front. John Hunter constructed double-chambered bellows, one chamber being used for filling the lungs and the other chamber for suction.* Hunter recommended the use of oxygen for inflation—discovered only two years previously by Priestly. Monro secundus also constructed a double-chambered apparatus; his bellows, however, were too large; the chambers had a capacity of 1500 cubic centimeters. The bellows, which were later accepted by The Royal Humane Society, were designed by Charles Kyte and had a capacity of only 500 cubic centimeters. The nozzle of the tube connected with the bellows was introduced into one nostril while the other nos-

* The invention of an apparatus for artificial respiration in which the inspiration is accomplished by insufflation and the expiration by suction has been in recent years usually ascribed to the noted Viennese pharmacologist, H. H. Meyer. As we see, this form of apparatus was invented and used by John Hunter at least 140 years before. See Keith.¹

tril, as well as the mouth, were kept closed, and the cricoid was lightly pressed backwards to prevent the insufflated air from passing down into the esophagus. Goodwin discovered that in the apparently dead the tongue falls backwards; the insufflation of air might therefore rather assist in occluding the entrance into the larynx by pressing down the tongue upon the epiglottis. Monro overcame this difficulty "by passing the catheter into the larynx by way of the mouth."* "From 1782 onwards bellows were recommended by The Royal Humane Society as the best means for artificial respiration. . . . For 40 years no one had a word to say against them; they were used in every country." However, in the twenties of the nineteenth century Benjamin Brody began to maintain that artificial respiration was only a secondary means in the resuscitation of the apparently dead, and in 1829 Leroy d'Etiolles stated in a memoir that it was possible to kill an animal by suddenly inflating its lungs. "Leroy's experiments alarmed The Royal Humane Society . . . the bellows fell into disgrace and in 1837 it disappeared from the list of methods recommended by the society." Keith tells us that "during the period in which artificial respiration was in abeyance the chief treatment lay in the application of warmth, treatment commencing with the immersion of a patient in a bath at about 100° F." The following data, taken from one of Keith's tables, present us with a bewildering illustration. From 1795 to 1811, when warmth and inflation (bellows) were used, there were as many as 54.8 per cent. unsuccessful cases; from 1832 to 1851, when warmth and friction were used, there were only 10 per cent. unsuccessful cases. Is this striking contrast merely an outcome of the familiar unreliableness of information derived from statistical data, or is it due to the fact that inflation of the lungs by bellows is indeed a very dangerous procedure?

As stated above, the year 1856 introduced the second period in the history of resuscitation. In contrast to the foregoing decades, artificial respiration became practically the only method of resuscitation. However, the character of the artificial respiration practised nearly exclusively in the greatest part of the second period, assumed a dif-

* More than a hundred years later we find in the medical literature several living writers claiming priority for the invention of this procedure.

ferent aspect. While in the first period inspirations were produced by *inflation*, in the new methods of artificial respiration, the inspiration was accomplished by *aspiration*, and the procedures, which are expected to bring about artificial respiration, consisted in manual handling of the victim. These methods of artificial respiration possess the undoubted advantage that no special apparatus are required for their performance, and that the manipulations can be started immediately on discovery of the victim. Some sort of movements of the chest and abdomen were undoubtedly practised ever since attempts of resuscitation were made. But the movements we are speaking of here are of an orderly, systematic character, based upon more or less well founded scientific observations.

These movements may be divided into three classes: 1. Movements which produce originally active expiration, while the inspiration is accomplished passively. 2. Movements which actively produce inspiration, while the expiration is accomplished passively. 3. Active movements producing the inspiration as well as the expiration. This last mode of respiration was the first new form which was introduced in 1856 by the celebrated physiologist Marshall Hall and which was termed by him the "postural method." The subject was first placed in the prone position and pressed upon the back, causing thereby an active expiration; it was then turned over on the side and the shoulder was raised, which brings about an active inspiration. Soon after Hall's communication was made, Sylvester introduced the method which is now known by his name. The subject is kept in the supine position and both arms are strongly raised above the head, thus stretching the pectoral muscles and causing hereby an active inspiration. The expiration is accomplished passively by returning the arms to the side of the subject. This method was recommended by the first Committee on Resuscitation appointed by The Royal Humane Society in 1861, and it is still largely in use in many parts of the world. In Germany it was adopted with a modification by Frosch: instead of returning the arms to the side of the patient, they are pressed on the cartilaginous part of the thoracic ribs, causing hereby some degree of active expiration. I wish to add that the last-mentioned procedure may possibly have the further advantage *that it is capable of producing some stimulation of the heart.* By the antago-

nists of the Sylvester method, it is accused that in some instances it has caused fracture of the ribs and even rupture of the liver. About 1871, Howard, a police surgeon of New York, suggested another method, namely, to place the subject in the supine posture, supported by a pillow, to apply the palms of the hands on the prominent parts under the subcostal margins, and to force the abdominal contents upward; thus producing active expiration. The inspiration is produced passively by the recoil of the ribs and the return of the abdominal contents to their original place. The method did not find favor, and it even has been alleged that rupture of the liver is apt to follow a too vigorous use of the Howard method.

In 1899 the Royal Medical and Chirurgical Society instituted its fourth committee. "Professor E. A. Schäfer was a member, but ultimately became the committee, and his report, read in 1903, is one of the most important contributions ever made to the literature of resuscitation" (Keith). The method which he recommended is now known by his name or as the "prone method." The subject is placed face downwards, the chest resting directly on the floor. The operator kneels astride the prone subject and places his hands over the lower ribs and the lumbar parts on each side of the spine. To produce the expiratory movement the operator, keeping his arms outstretched, brings the weight of his body on the lower dorsal region of the patient; the abdominal viscera are hereby powerfully compressed and forced against the diaphragm and lungs, which are thus brought into a position of ultra expiration. The recoil which occurs when the operator's weight is withdrawn causes an inspiration. In principle Schäfer's method is identical with that of Howard, except that it is easier to perform and has the important advantage that, the subject being in a prone position, the tongue falls forward and thus does not interfere with the entrance of air into the larynx.*

Some eight years ago a committee appointed by The Royal Society of Medicine recommended Schäfer's method in preference to that of Marshall Hall or Sylvester. Nevertheless, The Royal Society and

* Several years ago in a conversation which we had in London, Professor Schäfer remarked that "if he were now confronted with the task of resuscitation he would kneel astride over the subject and perform the simple motions of horseback riding without employing his hands and arms at all."

other life-saving associations still give, I believe, preference to the Sylvester method.

In the United States two commissions* investigated the methods of resuscitation. Both came to the conclusion that Schäfer's method is preferable to that of Sylvester.

Before mentioning other methods of artificial respiration employed in the last two or three decades in resuscitation, I wish to refer here to Laborde's method of traction of the tongue, although it can hardly be considered as a method of artificial respiration. There is no doubt that in some instances, especially in accidents from anesthesia, a few tractions of the tongue may reestablish respiration. It is evident that in most of these cases the aid is rendered by lifting the epiglottis away from the entrance into the larynx. However, it is not this effect which Laborde had in mind. He states that he succeeded by his method in resuscitating from drowning even after there was no respiration for one hour. Laborde believes that the favorable effect is due to reflex action through the glossopharyngeal and other nerves. Soon after Laborde's communication there were some favorable reports from the use of this method, especially in France. However, in recent years I have not come across any mention of this method in the medical literature.

Besides the previously mentioned manual methods in the last three decades a goodly number of procedures were proposed, and partly came into use, which collectively may be designated as mechanical methods of artificial respiration. Fell (1887)² was probably the first one to use successfully in human beings (in cases of poisoning) the method usually employed in the physiological laboratories (trache-

* On the first commission Drs. Cannon, Crile, Yandell Henderson, and Meltzer represented the American Medical Association; Dr. Spitzka and Mr. Elgin represented the National Electric Light Association, and Drs. Kennelly and Elihu Thomson represented the General Electric Company. Dr. Cannon was elected chairman and Mr. W. D. Weaver, then editor of the *Electrical World*, was elected secretary of the commission. Mr. Elgin, past president of the National Electric Light Association, deserves special credit for the creation and success of this committee. The second commission was appointed by the Bureau of Mines of the Department of the Interior and consisted of Drs. Cannon, Crile, Erlanger, Henderson, and Meltzer; the report of this commission is known as "Technical Paper No. 77."

otomy and bellows). Later Fell recommended the use of a mask in place of a tracheotomy tube. Northrup (1896)³ advocated the use of a modified O'Dwyer tube connected with bellows (Fell-O'Dwyer apparatus). At about the same time several French investigators attempted to devise methods of artificial respiration by the aid of mechanical means, among whom Tuffier and Hallion, and Doyen deserve to be especially mentioned. Doyen's apparatus consisted of "duplex" bellows (for insufflation and suction) attached to an intralaryngeal cannula. R. Matas (1902)⁴ constructed an apparatus in which a modified O'Dwyer tube was connected with an automatically acting pump. The pump contained originally two independent metal cylinders for inspiration and aspiration. However, the first experiment made on a dog convinced Matas that the suction force, exercised by the aspirating cylinder, does damage to the lungs, and he eliminated that part from his apparatus.* In his efforts to apply the method of intubation to all cavities and tubes of the body Franz Kuhn became also active (in Germany) in introducing the method of intralaryngeal intubation as a means of resuscitation.

To the foregoing the statement is to be added that, as is generally known, in the last seven or eight years various efforts have been made in this country to introduce mechanical resuscitating machines. But I wish to point out that most of these efforts are to be distinguished from all foregoing activities by one deplorable characteristic: while the activities in the foregoing periods were stimulated practically solely by humanitarian and scientific purposes, nearly all the apparatus and machines which are being introduced at present, and which receive a lesser or greater unscientific publicity, have one unpleasant feature in common, namely, the plain and unveiled commercialism

* Courtois seems to have been the first one who as early as 1790 (*Jour. de med., chir. et pharm.*, Paris, LXXXII, p. 361) introduced the duplex metal cylinder as a means of inducing artificial respiration under the designation of "pompe apodpinque" (quoted from a pamphlet by Dr. William Harrison, *Pneumatry papers*, No. 1, Detroit, Mich., 1916). According to Harrison, Poe's Artificial Respirator, which is now extensively advertised by the Life Saving Devices Company under the name Lungmotor, is identical with Courtois' "pompe apodpinque." Harrison himself is now introducing an apparatus under the name of "Pneumatizer" which, according to the inventor, is free from the shortcomings of the Lungmotor.

which is evidently the sole motive for their construction. Their introduction to the public is accomplished by the usual methods by which commercial products try to make headway, namely, by a more or less loud advertising. It is rather a sad chapter in the history of resuscitation; but I do not intend to dwell upon its details.

II. Criteria for the Efficiency of the Methods of Treatment.

What evidence do we possess that certain methods which are employed for resuscitation are really efficient? As far as I know there are at present only three criteria which may serve as guides for our judgment.

Statistics.—For more than 100 years the character of the evidence was exclusively of a statistical nature, namely, that if a certain method, employed for resuscitation, had a high percentage of successes to its credit, it was considered as a proof that the method was indeed a useful one. But the statistics given in the above mentioned articles of Keith demonstrate at a glance the fallacies contained in this method. For instance, for many years “fumigation” (introduction of tobacco into the large bowel) seemed to be a most reliable method. Later, however, on the contrary, it was forbidden. At another period when only warmth and friction were employed for resuscitation, these procedures, judging from the statistical data, seemed to have given the best results. Then the various methods of artificial respiration had, as seen above, their “ups and downs” in the history of resuscitation. It is evident that the value of the reports of successful treatment of the apparently dead varied greatly with the character and judgment of the reporters and with the nature of a great many circumstances which were not taken into consideration. Uncritical, statistical data, as is now generally conceded, have not only no value, but are very often decidedly misleading. As to the statistical data collected for transparently unlaudable purposes, with which regrettably all branches of medicine in our country is overwhelmed, the term “misleading” is surely insufficient for their characterization.

Respiratory Volume.—“Science is measurement,” quotes Schäfer in his Harvey Lecture.⁶ The criterion which played and still plays a considerable rôle in the estimation of the value of the methods of manual artificial respiration consists in measuring the volume of the

air inspired and expired into a spirometer. This method was, I believe, introduced by Schäfer. At first he experimented on drowned dogs; but these experiments gave no satisfactory results, because drowned dogs recover often without artificial respiration, sometimes even after having been for five minutes under water, that is, without any respiration whatsoever. On the other hand, if the heart of drowned animals stops beating, as a rule none of the manual methods of artificial respiration, no matter how early begun, is capable of resuscitating them. The experiments with measuring the volume of air on dead human bodies also gave no satisfactory results on account of the various degrees of rigidity of the thorax. The most appropriate way of testing the manual methods of artificial respiration seemed to be to study the volume of inspired and expired air of living conscious human beings. By using this method Schäfer found that his prone method of artificial respiration gave a much larger volume than the supine or Sylvester method. However, it was soon discovered that the normal individual interferes involuntarily with the inspiration and expiration either by partly closing the glottis and thus reducing the amount of the expiratory and inspiratory air, or the volume of the respiratory air is influenced by the unconscious innervation of the respiratory muscles. Ploman⁶ carried out his studies on human beings who were previously trained not to participate in the respiration during the execution of any of the methods of artificial respiration. According to him, Schäfer's method gives definitely smaller volumina of respired air than the method of Sylvester and its modifications. It seemed, however, that even a previous training was not a sufficient guarantee that the individuals experimented upon do not interfere sometimes unconsciously with the course of the artificial respiration. Lilijenstrand, Wollin, and Nilsson⁷ tried to overcome the difficulties by studying the effect of artificial respiration on conscious *apneic* individuals. The apnea was brought on by a small series of deep respirations, and it is generally assumed that during a state of apnea the individual is completely relaxed and has no desire for breathing. According to these authors the respired air volumina obtained under any of the manual methods of artificial respiration are much smaller than any normal respirations, but they agree with Ploman that Schäfer's method gives a still smaller volume. Loewy, one of the most active investi-

gators in this line came out,⁸ however, with the claim, based upon experimental studies, that during apnea the individual is not completely relaxed, but, on the contrary, the respiratory muscles are in a state of tonus, which may mean that in this state the individual is rather more ready to react to the stimulations produced by artificial respiration.

As mentioned before, the apnea produced in the experiments of Lilijenstrand, Wollin, and Nilsson, and probably also in those of Loewy, were brought on by a small series of respirations. Yandel Henderson⁹ undertook a critical examination of Schäfer's and other methods of artificial respiration on students "who experienced after overventilating their lungs by two or three minutes of voluntary forced respiration a lack of desire to breathe (apnea), lasting from 40 to 80 seconds." He found that under these circumstances Schäfer's method gives a slightly larger volume than that of Sylvester, especially when Schäfer's method is carried out with "the arms stretched forward." But even in Henderson's investigations the respiratory volumina obtained under artificial respiration were about one-half or less than the one obtained in normal respirations.

However, no matter to which of the various claims greater weight is to be attached, it seems quite evident that the method of measuring the volumina of inspired and expired air is incapable of demonstrating that any of these methods is in a position to keep up the respiration for any length of time when the respiratory mechanism is in a state of complete failure. It seems that in the end it is not the experimental method of measuring the volumina of the respiratory air which influences the contending parties to take sides with one or the other method; it is again essentially the old method of making claims by statistical data which induces the followers of various methods to consider one or the other method of manual artificial respiration as the more efficient procedure to employ in resuscitation of the apparent dead.

Curarized Animals.—Several years ago the writer¹⁰ introduced a new method of testing whether a certain procedure of artificial respiration is indeed capable of keeping an individual with complete failure of the normal respiratory mechanism interminably, or at least for many hours, alive. The essential factor in this method consists in keeping the respiratory muscles of the animal completely paralyzed

by means of curare for as many hours as we wish to continue our experiment. The animals are at the same time anesthetized by ether, administered by means of the method of artificial respiration which is undergoing the test. When the method of artificial respiration is inefficient the circulation comes sooner or later to a complete standstill. When, however, the method is efficient the circulation can be kept practically in a uniformly normal state for many hours, although the animal remains completely paralyzed. At the end of such a successful experiment spontaneous respiration can frequently be restored to normal by means of an injection of physostigmin.

By means of this method my procedure for artificial respiration, which I am going to describe later, has been tested on hundreds of animals. By the same method I have tested the efficiency of the Sylvester method, of the Schäfer method, and of the pulmotor which was so much in vogue at that time. Regarding the manual methods my statements can be brief, and are as follows: The longest time the circulation of a curarized and anesthetized animal could be kept up by the Sylvester method was twelve minutes, even when the tongue was kept pulled out by means of a forceps. The experiments were made on dogs. By the Schäfer method, with the anterior extremities stretched forward, the shortest period during which the circulation was kept on going amounted to about eighteen minutes. The longest period (one dog) lasted thirty-one minutes. In this dog the manipulations were carried on with great energy, and at the autopsy it was found that the liver was ruptured.

The efficiency of the pulmotor, which was loaned to us by the Bureau of Mines, I have investigated on a large number of dogs and the results deserve a more extensive statement. The pulmotor was applied by means of a mask fitted specifically for each dog; the tongue was kept withdrawn. In some instances the trachea was connected with a manometer to establish the pressures during inspiration and expiration. In some instances the thorax was split transversely and the condition of the lungs and the heart was established by ocular observation. The external part of the pulmotor consists of an ingenious mechanism which provides automatically for inspiration and expiration. The apparatus is driven by the force of a specifically adopted oxygen tank. However, according to Haldane and Hender-

son's analysis of the air-oxygen mixture delivered by it, it showed only 26.75 per cent. oxygen.⁹ The experimental results were strikingly disappointing. In my experiments out of a large number of dogs the circulation of only one could be maintained for one hour. The balance of the experimental animals died sooner or later. In these animals the circulation stopped when they were left exclusively to the effects of the pulmotor. The manometer connected with the trachea showed, as a rule, a comparatively small pressure, the utmost 40 millimeters of water. When the thorax was kept open it was observed that practically without exception with the continuation of the use of the pulmotor the size of the lungs became gradually smaller and smaller. This could also be observed in young animals with normal thorax. When the pulmotor had been used for some time, the lungs, in many cases, presented an uneven appearance—small collapsed areas alternating with emphysematous ones. It seems that in general our experience agreed with the experience of Matas when he used an apparatus with separate chambers for inflation and suction. The success with the pulmotor was more satisfactory when it was connected directly with the trachea by means of a tracheotomy tube. The inefficiency of the pulmotor seems to be caused essentially by two elements. The first element is its automatic arrangement. While the air-oxygen passes from the nasopharynx into the trachea it seems to meet with some sort of obstacle which converts prematurely the inspiratory movement into an expiratory one. The result is an insufficient exchange of respiratory gases. The second deleterious element is the suction; an element inherent to other machines on the market. Apparently in many cases the suction closes most of the bronchioles before it succeeds in removing deleterious expiratory gases from the alveoli. In these instances the air within the dead space is caused to move, which simulates respiratory movements without producing an actual exchange of gases. As stated before, my experience in the experiments with the pulmotor (which in one case has been witnessed by Drs. Cannon and Henderson) coincides with that of Matas. In a recent paper by Yandell Henderson¹¹ he states that he is inclined to doubt "whether an active withdrawal of air with a pump of limited stroke has any very serious objections" and refers to his experiments "with two pumps arranged in a manner simi-

lar to the 'lungmotor' and worked quite violently;" he observed "no particular ill-effects of the lungs." Without entering into a discussion of the nature of Henderson's experiments which do not permit a comparison with the activities of the pulmotor and lungmotor, it is sufficient to recall the fact that in the experiments of Henderson the pumps were connected directly with the trachea, a condition which, as mentioned above, increases the value of the pulmotor.

III. The Author's Method of Pharyngeal Insufflation.

The most reliable method of artificial respiration is, in the author's opinion, the method of intratracheal insufflation introduced about seven years ago by Dr. Auer and the writer. The writer has kept many animals for more than 24 hours continually under ether administered by the method of intratracheal insufflation without causing any injury to them. Furthermore, in several cases of poisoned human beings without any respiration, intratracheal insufflation was kept up for more than 15 hours until the patients recovered. However, the introduction of the tube into the trachea requires some dexterity and practice, and any of the apparatus now on the market used for keeping up the intratracheal (or as it is now called by some writers, endotracheal) insufflation is usually expensive. It is therefore highly improbable that in most emergency cases an apparatus for intratracheal insufflation as well as an expert surgeon who knows how to introduce the tube and how to handle the apparatus will be just at hand. When I was made chairman of the subcommittee of the above-mentioned commissions to study the problem of artificial respiration by means of apparatus I developed the method of pharyngeal insufflation which worked infallibly in hundreds of cases of animals under the influence of curare and ether.¹² Later, when I attempted to use such an apparatus on dead human bodies I found that the method had to undergo some changes in order to be adaptable to the human being.¹³ Since the last publication I have made some advantageous changes in this apparatus and had several opportunities to test the efficiency of the apparatus in its present form on living human beings with completely paralyzed respiration. In the following I am going to describe the apparatus, its principles, and the procedure of its application.

From the data reported in the historical part it can be seen that the idea of producing artificial respiration by means of a pharyngeal or laryngeal tube connected with bellows is not new. Neither are the ideas new that too much pressure of the air insufflated into the lungs may do harm or that a good part of the insufflated air may enter into the stomach and the intestines. However, when I started developing my apparatus I was not yet familiar with these data. When I began to use a pharyngeal tube for artificial respiration I was con-



FIG. 1.—Showing an oxygen tank, the interpolated rubber bag, the respiratory valve in an inspiratory position, the T-tube, and the pharyngeal tube.

fronted with several difficulties: the insufflated air is liable to escape through the nose or backward through the mouth, or the air may enter chiefly into the stomach and from there into the intestines and do more harm than good; at any rate, perhaps only little of the insufflated air will enter the lungs and may be insufficient to produce a satisfactory respiration. In the last published article¹³ I described devices which should meet these difficulties; I shall discuss them again in this paper. Another serious obstacle to the use of pharyn-

geal insufflation was the possibility of driving air into the lungs under too much pressure so that it may injure these organs. To meet this possible danger I suggested the interpolation of a mercurial valve into the tube which connects the respiratory valve with the foot bellows, the mercurial valve being arranged for about 20 millimeters pressure. This mercurial valve, however, was found to be, for reasons which I do not need to discuss here, a cumbersome and undesirable device. Another unsatisfactory condition seemed to come from the direct connection of the bellows with the respiratory valve; the air from the foot bellows is practically not continuous and the inspiration develops only gradually to the desirable pressure. On account of these defects two advantageous changes were introduced in the present apparatus, the entire details of which I am now going to describe.

First, *the foot bellows*, they need no special description. It is hardly necessary to state that the required inspiratory pressure could be obtained either from an oxygen tank or from a tube coming from a constant pressure somewhere in the hospital or other building. The bellows are merely recommended on account of their comparative cheapness and the convenience with which they can be transported to any place where they may be needed. Second, *the respiratory valve*. It is a small tube which may be conveniently kept in the hand when using it; the tube has a protruding movable ring into which the thumb fits. When the thumb moves the ring to the right side the driven air or oxygen pass through the respiratory valve, and, as will be seen later, pass further into the pharynx and into the lungs. When the ring is moved to the left side the escape from the respiratory valve is prevented and the air or oxygen accumulate meanwhile in the large bag interpolated between the source of pressure and the respiratory valve, while an aperture appears above the ring for the escape of the expiratory air from the lungs. (See Fig. 1.) In the tubing connecting the bellows with the respiratory valve a large rubber bag is interpolated. The presence of the bag offers the advantage that during the expiratory phase the air or oxygen may accumulate in that bag and be ready for a full inspiratory blast as soon as the thumb moves the ring to the right side. Third, *the pharyngeal tube*. This tube has a flat surface at its lower side which rests on the tongue and a curved

surface on its upper side. At the pharyngeal end of the tube the upper surface is longer than the lower one. The external end of the tube has a protrusion with a neck for connection with the respiratory valve and an opening through which a stomach tube may be pushed down through the esophagus into the stomach. When no tube is in use this opening is closed with a movable plate. (See Fig. 2.) Fourth, a *T-tube* which is interpolated between the respiratory valve and the pharyngeal tube. The T-tube carries on its rubber end a clamp screw, which, when not screwed down, permits most of the air to escape through the tube, while, on the other hand, by *gradually* screwing down the clamp upon the rubber tube the amount of air entering the pharyngeal tube will gradually increase. (See Figs. 1 and 3.) This T-tube arrangement supplants, as it will be shown later,



FIG. 2.—Showing an enlarged pharyngeal tube (Ph.T.) with its various particulars, and a stomach tube (S.T.) in it.

the mercury valve of the earlier form of apparatus and is used with great advantage for the same purpose, namely, to prevent the harm of an excessive pressure. Fifth, a *padded wooden board*, to be used for compressing the abdomen by means of belts. (See Fig. 4.)

The rubber tubing used in this apparatus must have thick walls, so that it should not kink; metal and no glass tubes ought to be used for all other connections—they should not break when the apparatus is used in a great hurry and without special care. All these parts *ought to be kept connected and kept in readiness* in a handy small bag. In addition to the described apparatus the bag ought to contain, (1) a stomach tube which fits into the external opening of the pharyn-

geal tube; (2) an appropriate tongue forceps; (3) a roll of tape, and (4) a pair of scissors. Bellows, rubber tubing, etc., should be frequently examined for their efficient activity, in order that the apparatus should not fail when its application is needed in an emergency.*

When coming to a victim who requires immediate artificial respiration the order of the procedure should be as follows: First, the application of the abdominal board—in order to prevent the entrance of the insufflated air into the stomach and the intestines. Second, to pull out the tongue as far as possible by means of the forceps. Third, to insert the pharyngeal tube of the readily connected apparatus as deep into the pharynx as possible with the flat side of the tube on the tongue. The tongue should now be tied to the tube by means of tape—not too tight. The tying of the tongue has two purposes: (1) It prevents the falling back of the posterior end of the tongue and of the glottis, and (2) it keeps the pharyngeal tube in place. The working of the bellows with one foot, and the moving of the ring of the respiratory valve with the thumb of the right hand should be started immediately on tying the tongue to the pharyngeal tube. At the beginning of the procedure the T-tube should be kept open; it should soon be gradually screwed down until the thorax shows a distinct raising when turning the ring to the right and falling, when turning to the left. The heaving of the chest need not be too strong. The degree of the heaving can be readily controlled by means of the screw, which should be turned down gradually, and which will then be capable of accomplishing all the care which may be obtained from the use of a mercurial valve. Moving of the ring thirteen to fifteen times per minute will give a satisfactory respiration; or the operator may time the moving of the thumb by the rhythm of his own respirations. In case of need one individual who had some training may accomplish all three procedures and start the artificial respiration in less than one minute after finding the victim.

Should the respiratory failure take place while the abdomen is open, a condition in which the abdominal board cannot be applied,

* The apparatus ("Meltzer's pharyngeal insufflation apparatus" for artificial respiration in cases of emergency in man) can be obtained from George Tiemann & Co., 107 Park Row, New York City. It is not patented. Its cost is probably less than one-fifth of the patented apparatuses.

the plate at the outer end of the pharyngeal tube should be opened and the stomach tube pushed through the opening into the stomach: any surplus of air which may enter into the stomach will thus promptly

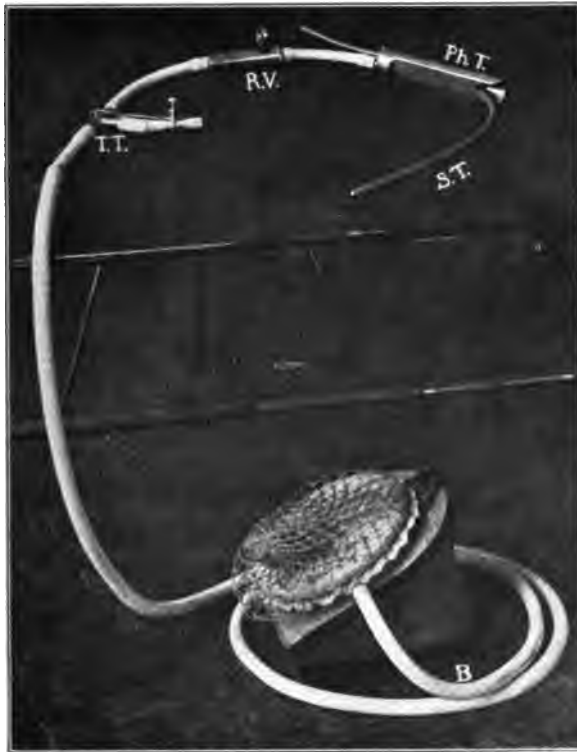


FIG. 3.—Showing an earlier arrangement of the apparatus. B., bellows; T.T., T-tube inserted between the bellows and the respiratory valves; R.V., respiratory valve; Ph.T., pharyngeal tube; S.T., stomach tube.

escape through the stomach tube. This device has been tested on many paralyzed animals and it was found thoroughly capable of properly supporting the function of respiration. The insertion of the stomach tube may be used even where the abdominal board is being applied; in fact, it may be saving time by introducing the stomach tube immediately on inserting the pharyngeal tube; the application of the abdominal board may be carried out later with greater leisure.

On the other hand, the application of the abdominal board has the further advantage that it drives a part of the blood contained in the splanchnic region into the heart and thus supports to a degree the failing circulation. (See Fig. 1 in my previous article.¹³)

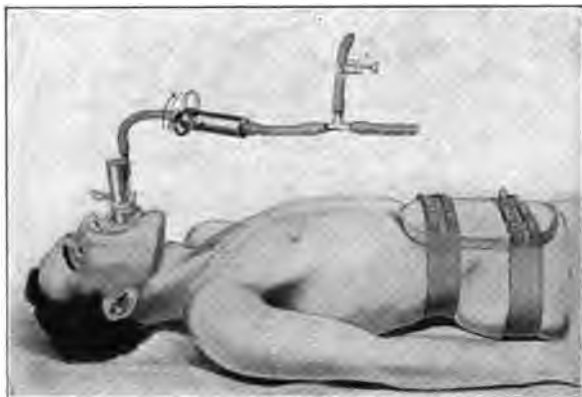


FIG. 4.—Showing the apparatus in position, except the bellows and an oxygen tank.

The size of the pharyngeal tube which goes with the apparatus is generally sufficient to prevent too much of an escape of the insufflated air through the mouth; furthermore, the prolongation of the curved side of the tube raises the soft palate and thus prevents the escape of air through the nose. However, we need not care about slight escapes of air, since the amount of insufflated air can be easily controlled by means of the T-tube and the clamp screw. In my experience, which extends to many hundreds of animals and a few human beings, a single trained individual is capable of working the bellows with one foot and using the respiratory valve with one hand, while the other hand controls the insufflated pressure by means of the T-tube. On various occasions (at the Army Medical School, Washington, at the Johns Hopkins Medical School, in various hospitals, etc.) this method of artificial respiration was demonstrated on curarized animals with the thorax wide open, the administration lasting as a rule, for several hours. The pharyngeal tube was often removed for a long time, so that the heart practically stopped beating, and it then

took only a very short time to start the artificial respiration and bring the heart to its previous normal beat. *At least in four instances the respiration was in complete abeyance in human beings for ten minutes or longer while the here described apparatus kept the heart going at a normal rate and the entire circulation in good condition.**

I wish, however, to state expressly that our test by which we gauge the value of apparatus or of manual methods of artificial respiration is a severe one. It presupposes that we always have to deal with victims whose respiratory mechanism is completely paralyzed and cannot be reached by any reflex stimulation. But there can be no doubt that the vitality of the nervous system of many victims, although they actually show no respiration, is not yet completely abolished and may therefore still be capable of recuperating sooner or later and of responding to some stimulation. In such cases even methods of artificial respiration of moderate efficiency may sometimes be capable of sustaining the life of the individual for a certain period, at least, until an efficient apparatus is procured. Furthermore surely there are cases in which the application for a few minutes only of even inefficient apparatus or methods may support the failing respiratory mechanism and thus help to restore the life of the victim. It is therefore proper to advise that in the absence of reliable and efficient apparatus for artificial respiration any type which is just at hand ought to be used—provided the apparatus does not possess the two above-mentioned dangerous elements, namely, *the automatically regulating inspiration and expiration and the special provision for expiration by means of suction.*

* To my mind the apparatus which is here described is practically the most efficient one for the execution of artificial respiration in cases of emergency. It is absolutely reliable; it is very easy to handle and is comparatively inexpensive. My statements which are here made are based upon personal observations. I have, of course, very few opportunities of testing it upon human beings. I hardly need to state that I have no other motive for pleading for the introduction of this apparatus into medical and Samaritan practice than a scientific and a humanitarian interest. The manufacturers are spending immense sums of money for advertising and popularization of their machines. Their agents have no other means of demonstrating the possible usefulness of their respective apparatus than by showing its action upon a rubber bag; I need not discuss here the unreliableness of any of these demonstrations; neither do I need to discuss the inefficiency of the evidence derived from testimonies of some physicians.

However, it is rather probable that in most instances no kind of apparatus for artificial respiration will be at hand when an accident has happened. It ought therefore to be the rule that in each and every case a *manual* method of artificial respiration must be instituted immediately on discovery of an individual who shows no spontaneous respiration. As we have seen above, both methods which are now in vogue, namely, that of Sylvester and of Schäfer, may prolong, to some degree, the life of curarized animals. On the other hand, it was found that the life of curarized animals was sustained longer by using the Schäfer method than by the method of Sylvester. But we must keep in mind that all these methods, simple as they may appear, require some experience for their proper execution. I shall therefore suggest that in rendering first-aid to accident cases the method of immediate choice should be the one for which there is an operator present who possesses special experience in just that method. In other words, the Sylvester method should be given the preference if the men who are to render the first-aid are more experienced in this method than in that of Schäfer; one is capable of rendering better aid with a method with which he is familiar than with one which he has never used before. However, I shall here go on record, as I did before in the report of the mentioned commissions, that *generally the Schäfer method is preferable* to that of the Sylvester method.

In using the prone or Schäfer method I wish to remind here of the remark made to me by Professor Schäfer several years ago and which I have quoted above, namely, that "if he were now confronted with the task of resuscitation he would kneel astride over the subject and perform the simple motions of horse-back riding without employing his hands and arms at all." This simple manner of employing the prone method could be readily combined with the use of the author's pharyngeal insufflation apparatus, and the operator who is performing the Schäfer method could at the same time manipulate with one hand the respiratory valve and with the other hand regulate, when necessary, the T-tube. *But the operator must learn to move the ring of the valve to the left (expiration) synchronously with throwing his body downward upon the individual and to move his thumb to the right (inspiration) simultaneously with the raising of his body from the individual.*

We have so far discussed the present methods of resuscitation from the point of view of artificial respiration. But life depends at least as much upon the proper circulation as it depends upon an efficient respiration, and here we must confess that for the present we have no method which would act resuscitatingly upon the circulation, at least no method which could in any way be compared with the methods at our disposal for the performance of artificial respiration. However, it is worth while mentioning the few inefficient procedures at our disposal by which we may assist in restoring the circulation.

1. We ought to keep in mind that in a great many instances the very act of performing proper artificial respiration may render a direct service to the circulation. At a certain low ebb of life the failure of the circulation and that of the respiration form together a vicious circle: when the respiration is inefficient the blood which circulates through the coronary arteries is insufficiently oxygenated, whereby the contractility and conductivity of the heart muscles become greatly reduced and the heart commences to fail. This in turn retards and lowers the circulation within the medulla oblongata, whereby the respiratory (and the vasomotor) centers become asphyxiated. Thus the deleteriousness of the circle continues to increase until both functions finally fail. If before the final failure we could cut through the vicious circle, we may succeed in reversing the process to such a degree as to restore both functions. By employing an efficient method of artificial respiration we may therefore be capable also of helping the failing circulation and thus restore the life of the sinking individual. Thus efficient artificial respiration may be considered as a contributing factor in restoring the failing circulation.

2. Massage is sometimes an invaluable procedure in restoring the heart's action. Massage of the heart is frequently executed by rhythmically striking the thorax at the region of the heart. If employing the Sylvester method for artificial respiration a heart massage can be exercised when returning the arms to the thorax. In some cases massage of the heart was performed from the abdominal cavity through the diaphragm. We shall not dwell here on further details of that method.

3. The injection of adrenalin by the intravenous or intracarotid methods (Crile) may prove as a useful remedy in restoring the heart's

action. In experimental work its good effect has been frequently observed.

4. The compression of the abdomen, as we employ it in our method of artificial respiration, drives, as stated before, a part of the blood from the splanchnic region into the heart and from there into the general circulation. In experimental work we have frequently observed that this method is capable of increasing the blood pressure by 20 or 30 mm. of mercury.

The old method of employing various forms of stimulations in resuscitating the apparently dead ought not to be neglected. While it is quite probable that few of these methods, when used alone, have ever restored life, they may nevertheless render some service when used in combination with more efficient methods. The application of heat, friction, the mechanical and chemical stimulations of the mucous membrane of the nose, may sometimes render contributory service. Stimulation of the precordial or pudic regions or of the soles of the feet frequently exert an effect upon the respiration (*i.e.*, if the respiratory mechanism is not entirely paralyzed) and may at the same time affect the vasomotor centers and perhaps also the heart by the way of the accelerator nerves. While all first-aid men must be impressed with the fact that their first attention must be given to the respiration, it is not necessary to impress them that all the other means have no value. Anything which contributes to the resuscitation in the smallest degree should not be omitted, provided that the essential procedures are well taken care of.

Before concluding, I wish to add the following few brief remarks: As it will be seen in my present paper, I omitted the advice given in previous papers, namely, that in the absence of a proper pharyngeal tube to use a mask. After a long experience and a further analysis of the conditions, I came to the conclusion that a mask is liable to do a great deal of harm by driving infectious material, usually present in the nose and the pharynx, directly into the lungs and thus produce a fatal inflammation of that organ. By using the pharyngeal tube that danger is reduced to a minimum. But I wish to emphasize here that even in this method a minimal danger is present, and therefore recommend the employment of this method of artificial respiration *only in emergency cases*. When we find an individual apparently dead

we pay little attention to the possibility that our attempts of resuscitation may lead later to some disease of the victim; we would rather see the individual alive, although temporarily sick, than see him dead. Nevertheless, we should reduce this possible danger to its minimum.

In connection with the foregoing remark I wish to make the following brief statement. On account of the difficulties which some anesthetists meet with the introduction of the tube into the trachea, they began to advocate what they term intrapharyngeal or endopharyngeal anesthesia. I wish to go here on record that in my opinion this is definitely a retrograd step. I am not familiar with the details of the methods which some of the advocates of endopharyngeal anesthesia employ. From a general reading of, and occasional listening to, a discussion of that subject, I am under the impression that the methods which they employ are incapable of keeping up the respiration in case this function should fail. It seems to me that anesthetists are not familiar with the facts (or, at least, pay very little attention to it) *that anesthesia reduces greatly the efficiency of the normal respiration.* That patients, nevertheless, survive the anesthetic procedure is due to the fact that the normal function is well provided with *factors of safety*, so that if nothing unusual happens life may continue even with a low degree of respiratory exchange of gases. With my method of pharyngeal insufflation, respiration can be kept up for many hours in an efficient way, but, nevertheless, I advocate its use only in cases of emergency—for the above mentioned reason. From unpublished statements I learned that post-operative pneumonias are not an infrequent occurrence after the use of pharyngeal anesthesia. The contrast between the intrapharyngeal and intratracheal insufflation ought to be evident at a glance. In the intratracheal insufflation all foreign substances are driven backward. In pharyngeal insufflation, on the contrary, foreign substances are driven directly into the lungs. Furthermore, as a method of anesthesia, I discarded the intra-pharyngeal insufflation very early¹² on account of the profound effect which it produces upon the animal; in several instances I had to continue the insufflation without anesthesia for the purpose of administering artificial respiration for longer than half an hour before the animal started breathing spontaneously.

I wish therefore to be explicit in my statement. I recommend the

use of my apparatus for pharyngeal insufflation only for artificial respiration in cases of emergency and I advise especially against its use as a routine method for the administration of anesthesia.

Finally, I wish to call the attention of authorities who are frequently confronted with the need of artificial respiration (superintendents of hospitals, health and police departments, first-aid stations, etc.) that in the method which I described here we possess a reliable and easy to handle procedure and a comparatively very cheap resuscitating apparatus. It is highly desirable that the men who may have to perform the resuscitation by means of this apparatus, should familiarize themselves with the apparatus and the mode of procedure. The apparatus and its use are very simple; but even the most simple thing requires some practice in handling it properly.

How to Employ Melzer's Pharyngeal Insufflation Apparatus in Cases of Emergency.

On finding an unconscious individual, without any, or with very insufficient respiration, employ immediately the apparatus (when at hand) in the following order:

1. Put the board on the abdomen (over the clothes) and compress it by the hand; the tightening of the belts can be finished later.
2. After examining mouth and throat for the presence of tobacco, false teeth, etc., pull out the tongue by means of the forceps (present in the hand bag) and insert the pharyngeal tube (the smooth side of the tube resting on the tongue) until it reaches the posterior wall of the pharynx; the tying of the tongue to the pharyngeal tube can be done later.
3. Start immediately working the bellows with one foot and moving the ring of the respiratory valve from right to left and from left to right (respiratory cycle) about twelve cycles per minute.
4. Start immediately to screw down the rubber tubing (which should be open at the beginning) attached to the T tube, while watching the chest for heaving (respirations); as soon as there is a definite heaving discontinue screwing the clamp screw. (In order that the screw should not fall off, it should be attached by means of a piece of cord to the metal T-tube.)

5. Now tighten the board, and tie the tongue (not too tight) to the pharyngeal tube; the forceps may be then taken off. (If the respiration appears insufficient it is usually due to the falling back of the tongue; it should be pulled out again. If there is a steady tendency to fall back, either the tying should be made more tight or the tongue must be kept pulled out constantly by means of the forceps.)

6. After the respiration has been continued for about a minute turn the plate on the outside end of the pharyngeal tube and insert into the opening the stomach tube (present in the bag) and push it down into the stomach, or at least until it is noticed that air escapes from the external end of the tube. Throughout these manipulations the working of the bellows and of the respiratory valve should not be discontinued.

If several persons are present, and willing and capable to lend a hand, the very first act should be the attempt of resuscitation by means of the Sylvester method, while another person compresses the abdomen and tightens the belts for the abdominal board. As soon as this is done the Sylvester method should be discontinued and the pharyngeal tube inserted; the rest should be continued as outlined above. During the performance of artificial respiration other individuals may remove the clothing and various forms of stimulation may be applied; but the artificial respiration should under no circumstances be discontinued to allow any other performance.

The various parts of the apparatus should be always kept connected and frequently examined for their efficient working. The apparatus as well as the loose parts mentioned in the text must be kept collected in the bag ready for immediate work when required.

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EXPERIMENTS ON THE CAUSATION AND AMELIORATION OF ADRENALIN PULMONARY EDEMA.

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PLATES 14 TO 16.

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During an investigation of the absorption of drugs¹ by the pulmonary air passages of the rabbit we observed that an intratracheal injection of adrenalin, after a double vagotomy, apparently produced a fulminant pulmonary edema much more readily than when the vagi were intact. In the present paper we shall report a study of this subject, together with other series of experiments, which finally led to the recognition of a practically ignored respiratory factor in the production of adrenalin pulmonary edema.

That adrenalin may cause pulmonary edema in the normal rabbit was noted early in the experimental investigation of this substance. Bouchard and Claude² in 1902 observed pulmonary edema and death in the normal rabbit after the intravenous injection of adrenalin, an observation abundantly corroborated. Pulmonary edema in the rabbit may also be obtained when the adrenalin is injected subcutaneously, though tremendous doses are then necessary; thus Battelli and Taramasio³ produced this effect with the subcutaneous injection of 10 mg. of adrenalin per kilo.

The facilitating action of vagus section upon the occurrence of adrenalin pulmonary edema in the rabbit has not to our knowledge been observed before. In artificially induced hydremic plethora, however, the vagi, according to Kraus,⁴ play in this respect a decisive part.

¹ Auer, J., and Gates, F. L., *J. Exp. Med.*, 1916, xxiii, 757.

² Bouchard, C., and Claude, H., *Compt. rend. Acad.*, 1902, cxxv, 928.

³ Battelli, F., and Taramasio, P., *Compt. rend. Soc. biol.*, 1902, liv, 815.

⁴ Kraus, F., *Z. exp. Path. u. Therap.*, 1913, xiv, 402.

EXPERIMENTAL.

Methods.

Rabbits only were used in this investigation. The animals were placed on an electric warming pad and ether anesthesia was employed for operative procedures. A wide glass tube about 2 cm. long was tied into the trachea. When blood pressure was recorded the right carotid artery was connected to the mercury manometer by tubing filled with half saturated sodium sulfate. When the lungs and heart were inspected the sternum was split longitudinally; usually both pleural sacs were opened. Intratracheal insufflation was then started so that the lungs were always moderately distended. The vagi, when sectioned, were divided in the neck.

The adrenalin employed was the ordinary commercial product obtained in the market, and was slowly injected by syringe through the tracheal tube. The dose was usually 0.25 cc. per kilo of body weight; occasionally more or less was used.

When atropine was administered, this drug was usually injected intratracheally; in a few instances the atropine was injected intramuscularly.

As criteria for the presence of pulmonary edema we utilized the appearance of foam in the trachea and the autopsy findings. As a rule, the medulla was punctured in the survivors 30 minutes after the injection; in some instances 1 to 3 hours were allowed to elapse.

Although the appearance of the typical tracheal foam is positive proof of the existence of pulmonary edema, yet its absence by no means indicates the absence of pulmonary edema. Râles may be audible to the unaided ear and easily felt by the fingers and yet the trachea may remain perfectly clear of foam. This happens especially when the injected animals do not struggle; there is then no mechanical compression of the lungs and the foam and fluid remain in the lower air passages. For this reason we killed the surviving animals after a period of time by medullary puncture and at once examined the lungs.

Several series of experiments were carried out. In the first series both vagi were divided and after not less than 10 minutes the adrenalin was injected. The controls for this series were normal animals with intact vagi, but in many of them the adrenalin was injected repeatedly, thus rendering the control test especially severe.

In another series with divided vagi, the behavior of the heart was examined by inspection, the sternum being split lengthwise and the pericardial sac opened. Artificial respiration was instituted before the lungs were exposed. The adrenalin was always administered repeatedly. In a subsidiary series artificial respiration, with and

without ether, was given to rabbits with chests intact but with vagi divided.

In still another series the vagi were again divided, but atropine was administered before the injection of adrenalin.

In a final series the effect of tracheal stenosis upon the production of pulmonary edema in normal rabbits (vagi intact) was tested.

Results in Series with Divided Vagi.

The vagi were divided before the adrenalin was injected. The average dose was 0.25 cc. per kilo, and as a rule only one injection was given. The following protocol will give a clear picture of a typical experiment.

Rabbit 1.—Gray male; weight 1,740 gm.

10.45. On electric pad at medium.

10.58. Start ether.

11.02. Operation completed; both vagi divided, cannula in trachea. Stop ether.

11.08. Respiration rapid, easy. No inspiratory stoppage. Heart moderately rapid, regular, good strength.

11.10. Change pad to low.

+11.12. 0.52 cc. adrenalin into trachea (0.3 cc. per kilo); no loss.

11.13. Respiration more shallow and more rapid.

11.13½. Respiration slowed, deeper, with retraction of costal margin; respiration stopped.

11.14½. Respiration starts, slow, deep, with retraction of costal margins. Heart very rapid, apparently regular, good strength.

11.15. Struggle; pink foam in tracheal tube.

11.15½. Pink foam pouring from tracheal tube.

11.16. Struggle, marked outflow of pink foam. Heart apparently regular, rapid (palpation).

11.17. Respiration rapid; strong retraction of costal margin.

11.18. Convulsive struggles; pink foam and fluid pouring from tracheal tube. No heart palpable. Occasional gasp. Pupils wide.

11.19. No respiration. No heart palpable.

Autopsy at once. No excess fluid in peritoneal cavity. Well marked peristalsis of small gut and cecum; cecum full of gas. Spleen and liver congested. Kidneys apparently normal. No hemorrhages in diaphragm. Lungs do not collapse. Heart large, fills entire pericardial sac. Auricles and ventricles dilated. Heart flabby, large, with no hemorrhages on pericardial or endocardial surfaces. No clot in pulmonary artery or aorta. Lung and trachea excised;

lungs large, erect, heavy, surfaces covered with many small hemorrhages, often confluent. Lower lobes show most hemorrhages; bluish black-red color; upper lobes pink. In trachea pink foam and white foam, alternately in layers. On section, lower lobes extremely juicy, like a wet sponge; not much air, chiefly red fluid with some foam. Upper lobes contain a large amount of white foam, not much fluid; not heavy like lower lobes (Fig. 2).

Of 27 rabbits whose vagi had been divided, 21 showed on autopsy a marked pulmonary edema of the general type described in the preceding protocol. 14 died within 20 minutes of the single intratracheal injection of adrenalin, and of these 10 died within 10 minutes, showing the general symptoms noted above. In only 6 did the autopsy show slight or no edema. Thus in about 78 per cent (21 out of 27) of the animals a single intratracheal injection of a moderate dose of adrenalin caused a marked pulmonary edema.

Control Series with Vagi Intact.

In this group of sixteen animals the adrenalin was usually injected repeatedly into the trachea, the time interval varying between 4 to 20 minutes. The dosage was usually 0.25 cc. per kilo. In addition to the intratracheal injection a number of the rabbits received intramuscular injections of adrenalin.⁵ This series therefore forms a severe control for the group with divided vagi.

The results are briefly as follows: None of the rabbits, in spite of the repeated injections, died within 20 minutes; 3 succumbed after 40 to 45 minutes. The survivors (13) were either killed by medullary puncture 15 to 60 minutes after the first dose of adrenalin or were etherized after 15 minutes and the heart was clamped off. Autopsy revealed in no instance a pulmonary edema of the degree which was found in the series with divided vagi. Pulmonary edema, however, was present to some degree in the lungs of most of the animals; in 6 rabbits the edema was moderate, in the remaining 6 it was slight; one autopsy record was lost.

A comparison of the two series shows clearly that the intratracheal injection of adrenalin exerts a much severer effect in vagotomized

⁵ Specimen protocols in tabular form will be found in our previous article, Auer and Gates,¹ pp. 760-761.

than in normal animals. Thus 14 out of 21 vagotomized animals died acutely in 20 minutes after a single intratracheal injection of about 0.25 cc. of adrenalin per kilo, while only 3 out of 16 normal animals succumbed within 40 minutes, although the normal animals received the adrenalin repeatedly. In addition it must be emphasized that the degree of pulmonary edema was much greater in the vagotomized series than in the control series whose vagi were intact.

Behavior of the Heart.—In a number of experiments where the blood pressure was recorded, it was observed repeatedly that the blood pressure at one time or another after the adrenalin injection showed sudden, profound drops varying in duration from 10 seconds to 3 minutes associated usually with convulsions after the low pressure level had been attained. Foam in the trachea was noted in some cases, especially those in which the low pressure level was maintained for some time. In Fig. 1, for example, foam first appeared in the trachea after the second series of convulsions following the second abrupt drop of blood pressure.

These abrupt drops of blood pressure were obtained not only in animals with intact vagi, but also in those whose vagi had been sectioned. The cause of these drops was therefore peripheral and probably located in the heart itself. In order to gain information on this point the heart was exposed in a series of seven etherized animals by splitting the sternum and opening the pericardial sac. Usually both pleural sacs were also opened, the respiration being maintained by intratracheal insufflation. In all except one animal the vagi were severed. A specimen protocol will illustrate the observations made and their time relations.

Rabbit 2.—Gray female; weight 1,885 gm.

10.20. On pad at low. Ether.

10.46. Operation finished: both vagi divided; cannula in trachea; chest split longitudinally to expose heart. Insufflation of air-ether with remissions; air catheter through tracheal cannula.

Change pad to medium. Heart beats regular, rapid after opening pericardium. Both pleural cavities open; lungs pink, good distension; spontaneous respiration present.

+ 10.50 0.47 cc. of adrenalin into tracheal cannula (air catheter temporarily withdrawn), 0.25 cc. per kilo.

10.51. Left ventricle beats half as fast as right ventricle, right auricle, and left auricle. Left ventricular contractions weaker than those of right.

10.54. Both ventricles beat synchronously, following auricles; left ventricle stronger beat than before. No foam in trachea.

11.01. Chambers beat synchronously; good strength.

+11.03½. 0.47 cc. of adrenalin, into trachea.

11.04. Left ventricle half as fast as right ventricle; left auricle very large now; both auricles and right ventricle beat at same rate; left ventricle at half that rate and contractions weak.

11.06. As before, but left ventricle beats more strongly.

11.06½. Left ventricle shows strong and weak beat alternately.

11.08. Left ventricle now beats at same rate as right ventricle; left auricle much smaller now.

Animal killed by clamping the heart. On excising the lungs, the right side is large, full, rounded, with no emphysematous blebs on surfaces; the left side collapses fully. Right lung shows a good amount of foam in the alveoli and large bronchi; the left lung exhibits only slight edema. The surfaces show a moderate number of pin-point hemorrhages. Heart beats vigorously after excision; there are numerous hemorrhages over the left and right ventricles. Left auricle shows extensive hemorrhage on inner surface. Right ventricular endocardium shows a fair number of hemorrhages, only a few in left ventricle. Heart swiftly passes into rigor.

The protocol given above does not, however, show all the gross changes which may be observed in a series of experiments by inspection of the heart. The change of the left ventricular rate to half of that of the right ventricle is usually preceded by a stage in which the left ventricle shows an alternation of a weak and a strong beat. Then the weak beat becomes no longer detectable to the eye and the left ventricle apparently beats at half the right ventricular rate. The left auricle usually beats at the same rate as the right half of the heart, but in one instance its rate was that of the left ventricle; the right half of the heart thus contracted twice to one contraction of the left half. This alteration in the functional activity of the left ventricle was noted in six of the seven experiments. The duration varied from a few seconds to 3 minutes.

In a few instances the entire heart stopped for the length of a beat or two, which accounts for the abrupt blood pressure drops mentioned previously. The dilatation of the left auricle was often tremendous, and made this structure look like a bright red blister. This dilatation is not dependent upon section of the vagi, for a sim-

ilar large dilatation of the left auricle with marked swelling of the aorta and pulmonary artery was also noted in the single experiment of this series in which the vagi were intact.

The pulmonary artery may dilate tremendously after the intratracheal injection of adrenalin; the dilatation develops shortly after the injection and may last 7 minutes. This dilatation is just as marked during diastole of the right ventricle as during its systole. Dilatation of the pulmonary artery was noted in three experiments, not looked for in three, and not seen, though sought for, in one experiment.

The aorta dilated markedly shortly after the adrenalin injection in five experiments and its condition was not observed in two.

In two experiments the adrenalin seemed to exert a locally inhibiting effect upon the muscle of the ventricle. In one instance the upper half only of the right ventricle contracted during systole, the lower half dilating. In this experiment the state of the aorta and pulmonary arteries unfortunately was not observed, but the left auricle dilated enormously and the left ventricle showed the changes in beat already described.

In the second instance the musculature of the left ventricle was affected locally, and with each systole a prominent though small, bright red bulging appeared near the apex. In this animal the aorta and left auricle were strongly dilated; the left ventricle showed the changes in character and rate of beat described previously; the pulmonary artery was not observed. The pulmonary edema obtained in this animal was only slight.

In this series of experiments with double vagotomy and exposure of the heart under artificial respiration none of the animals died acutely, but all were killed after at least 20 minutes had elapsed, by clamping the heart or by medullary puncture. Moreover, the pulmonary edema observed in the excised lungs was only slight in four, and fair to moderate in the other three experiments.

In these experiments the right auricle and right ventricle showed no marked dilatation at any time, nor was any dilatation of the left ventricle observed which could account for the swelling of the left auricle by regurgitation through the mitral valves. On the contrary, the left ventricle was in a state of greater tone with small systolic and diastolic excursions, so that the distension of the left auricle was caused by the inability of this chamber to empty all the blood it received from the lungs into the tonically contracted left ventricle.

Although these experiments yielded interesting information concerning the activity of the heart after the repeated administration of adrenalin in vagotomized animals, the main result is the suggestive absence of any considerable pulmonary edema. This is the more interesting because all the conditions apparently favored the production of pulmonary edema: pressure in the pulmonary veins was surely raised because the dilatation of the left auricle shows that it received more blood than it could handle, so that back pressure resulted; the pressure in the pulmonary artery was also raised as shown by its dilatation; and still further proof of increased pressure in the pulmonary circulation is furnished by the development of hemorrhages during inspection of the lung.

As these experiments were necessarily carried out under artificial respiration, a small series of tests was made to study the effect of artificial respiration upon the production of pulmonary edema when the chest was intact. Four rabbits were utilized; their vagi were divided and intratracheal insufflation started with air-ether in various proportions; in one animal air only was given after the operation had been completed. The adrenalin dose was 0.25 cc. per kilo intratracheally, administered once. In this series one animal died 17 minutes after the injection without any convulsions or foam in the trachea. Death was due to too much ether; autopsy showed pregnancy and only a slight degree of pulmonary edema. The other three animals were killed by medullary puncture 25 minutes after the adrenalin was injected, and the lungs were examined at once. None of them showed a marked degree of pulmonary edema, but some was present in all, especially in the lower lobes.

These results thus indicate that artificial respiration has a restraining effect upon the formation of pulmonary edema, and corroborate a similar observation made by Emerson.⁶ How this protection is possibly exerted will be discussed later.

Tracheal Stenosis.

In another set of experiments the effect of an inspiratory decrease of pressure in the lung alveoli upon the production of pulmonary

⁶ Emerson, H., *Arch. Int. Med.*, 1909, iii, 368.

edema in animals with vagi intact was tested. This was accomplished by compressing the trachea with a clamp. The air now entered so slowly during inspiration that a rarefaction of the intrapulmonic air occurred, as was evidenced by the sinking in of the costal margins and of the lower part of the sternum. The lung alveoli thus must exert some suction during inspiration upon the capillaries in their walls, and this should aid in the production of an adrenalin pulmonary edema in normal rabbits (vagi intact).

Fifteen experiments were made, of which ten were controls. The adrenalin animals received 0.3 cc. of adrenalin per kilo intratracheally; after about 2 minutes the trachea was stenosed. About 15 minutes after the injection the animals were killed and the lungs examined at once.

In 5 rabbits where adrenalin had been injected intratracheally and the trachea stenosed, 4 exhibited a good or well marked pulmonary edema; in 1 the edema was slight. The edema, however, was never as great as that seen in vagotomized animals.

In 5 controls, adrenalin was injected in the same dosage, but the trachea was merely exposed, but not constricted. 4 of these rabbits showed slight or no edema, but the fifth exhibited a well marked pulmonary edema. This latter animal, however, was abnormal, for both peritoneal and pleural cavities contained a large amount of clear straw-colored fluid.

In the second set of controls no adrenalin was injected but the trachea was constricted and the animal killed by asphyxia 15 minutes after beginning the stenosis. Of these five animals, all showed either slight or no edema of the lungs. It is seen that stenosis of the trachea and final asphyxia do not suffice in the normal rabbit to bring about pulmonary edema.

This series of experiments thus shows clearly that the cupping action of the lung alveoli on the pulmonary capillaries during inspiration plays a part in the production of adrenalin pulmonary edema.

Distribution of the Pulmonary Edema.

The edema obtained after vagus section was not uniform in its distribution throughout the lung. Invariably the lower lobes were much juicier and more hemorrhagic than the middle and upper lobes. On section the heavy, lower

lobes resembled sponges soaked to saturation with a pink fluid; squeezing usually yielded little foam but much fluid. The middle and upper lobes showed chiefly white foam with little fluid; occasionally, however, the middle and upper lobes also contained much fluid.

Hemorrhages also were much more extensive over the surfaces of the lower lobes than over the upper lobes (Fig. 2). The outer third of the upper lobes was usually free from hemorrhages.

As a rule, the right and left halves of the lungs were equal in size, but in a number of instances the right side was appreciably larger than the left, and in some of these cases the pulmonary edema was also greater in the larger half.

Atropine Series.

This series of experiments was undertaken in order to test the effect of adrenalin after paralysis of the motor vagus endings. The animals were tracheotomized, the vagi divided, and atropine sulfate was injected in 1 per cent solution about 5 minutes before the adrenalin. In some cases the blood pressure was recorded. The thorax was intact, and no artificial respiration was given. The dose of atropine varied between 1 and 2 mg. per kilo; the amount of adrenalin was always 0.25 cc. per kilo, injected intratracheally. The atropine was injected intramuscularly in the first three animals but in the remaining seven it was administered intratracheally.

Of 3 animals which received atropine intramuscularly, 2 died acutely in 10 to 14 minutes after a single injection of adrenalin showing typical symptoms of pulmonary edema. The other rabbit, however, gave no clinical signs of pulmonary edema within 1 hour, during which he received not one but three doses of adrenalin, each 0.25 cc. per kilo, at 15 to 20 minute intervals. Death occurred after 2½ hours and the lungs exhibited a marked pulmonary edema. This last animal received a larger dose of atropine than the two preceding ones; the first two received 1 and 1.25 mg. respectively per kilo, while the third rabbit was injected with 1.5 mg. per kilo.

In the other seven experiments the atropine was injected intratracheally, the first dose tested being 1.25 mg. per kilo. This amount, in subsequent tests, was increased until 2 mg. per kilo were given. In this series the results were encouraging. 1 animal showed foam and fluid in the trachea, but was in fair condition 35 minutes after the adrenalin injection. The other 6 showed no clinical sign of edema,

the respiration was easy, no râles were palpable or audible, and no foam or fluid was ever seen during the 30 to 40 minutes of observation. All the animals were then killed by medullary puncture and the lungs examined: 5 animals showed slight or no edema, 1 showed moderate edema, and 1 exhibited a marked degree of pulmonary edema. This animal was the one in which foam and fluid issued from the trachea 15 minutes after the adrenalin injection.

Five control experiments without atropine were made with animals from the same lot. Of the 5, 2 died within 10 minutes and the remaining 3 succumbed within 20 minutes after the adrenalin injection. The lungs of all showed a marked degree of pulmonary edema. The differences between lungs of these two series is well brought out by Figs. 2 and 3.

The following table summarizes the results of the atropine series and its controls.

Atropine Series. Vagi Divided. All Injections Intratracheal.

No. of animals used.	Atropine per kilo.	Adrenalin per kilo.	Death in 10 min.	Death in 20 min.	Autopsy.
	mg.	cc.			
7	1.25-2.00	0.25	0	0	Slight or no pulmonary edema in 5 animals; moderate pulmonary edema in 1; marked pulmonary edema in 1.
5	None given.	0.25	2	3	Marked pulmonary edema in all.

The protective action of atropine against adrenalin pulmonary edema in normal animals has been noted by Biedl.⁷

DISCUSSION.

In the previous pages we have shown that adrenalin pulmonary edema occurs more frequently, in a more severe form, and after a smaller dose in rabbits if the vagi are sectioned previous to the intratracheal administration of the drug, than when the adrenalin is given in the same way to rabbits with vagi intact. It has been pointed

⁷ Biedl, A., *Innere Sekretion*, Berlin, 2nd edition, 1913, i, 522.

out that artificial respiration strongly reduces the degree of pulmonary edema after adrenalin when the vagi have been divided, and this result is obtained both with opened and intact chests. Data have been furnished which indicate that after adrenalin administered intratracheally the heart ventricles temporarily deliver unequal amounts of blood with each systole, the right ventricle delivering more blood than the left, shown by the tremendous dilatation of the left auricle and by the marked swelling of the pulmonary artery, which remained distended during diastole as well as systole; it has also been shown that adrenalin produces a temporary incoordination of the heart so that the left ventricle beats at one-half the rate of the right auricle and ventricle. Finally we have demonstrated that atropine markedly reduces the occurrence of pulmonary edema after vagus section and adrenalin.

What bearing do these facts have on the production of pulmonary edema? Leaving aside for the moment the striking adjuvant action of vagus section in the formation of pulmonary edema, it will be noted in the first place that the cardiac changes observed by us after the administration of adrenalin are in full accord with the fundamental postulate of Welch's theory^{*} for the production of a general acute pulmonary edema. It will be remembered that this is a disproportion between the ventricular outputs of such a character that the left ventricle expels less blood per systole than the right, thus producing an acute venous hyperemia of the lungs. In our experiments on rabbits whose hearts were exposed for inspection (artificial respiration) we saw this demand fulfilled: shortly after the intratracheal injection of adrenalin the pulmonary artery dilated widely and remained dilated during each diastole of the heart; at the same time the left auricle swelled tremendously and sooner or later the left ventricle showed a rate which was only half that of the right side of the heart, and its amplitude of contraction was small. These facts indicate unquestionably that the right heart is acting vigorously, that the arterial and venous pressures in the lung are

^{*} Welch, W. H., *Virchows Arch. path. Anat.*, 1878, lxxii, 375. See also his later presentation in S. J. Meltzer's Harrington Lectures on Edema, *Am. Med.*, 1904, viii, 37 of reprint.

raised, and finally that the left heart is surely expelling less blood per systole than the right.⁹

The physical conditions for the production of pulmonary edema demanded by Welch are therefore present, and yet the pulmonary edema which we obtained in this series with vagi divided, where the heart was exposed, under artificial respiration, was slight and even negligible when compared with the tremendous edema which resulted when the same dose of adrenalin was injected into animals (vagi divided) whose thorax was intact and which were breathing in the normal way. Artificial respiration thus seemed to be an inhibitory factor, a supposition which was strengthened by the results of another series of experiments with chest intact, where the vagi were divided and intratracheal air insufflation with rhythmical remissions of the pressure was given throughout. Here again no marked degree of pulmonary edema was observed after the injection of adrenalin, either clinically, or after killing the animal and examining the lungs. Moreover, there are statements in the literature which furnish direct and indirect evidence regarding the effect of artificial respiration on pulmonary edema.

Emerson⁶ in a brief note reports that gentle artificial respiration, with or without suction, produces an amelioration of the pulmonary edema in cats, caused by the intravenous injection of massive doses of adrenalin. That artificial respiration may even prevent adrenalin pulmonary edema in rabbits follows directly from a statement of Miller and Matthews¹⁰ that they were never able to obtain pulmonary edema in a considerable number of rabbits by injecting intravenously 0.5 to 2 cc. of 1:1,000 adrenalin. Now, 1 to 2 cc. of adrenalin, intravenously administered, are sure to cause pulmonary edema in a percentage of normal animals provided that the number tested is not too small, and Miller

⁹ While our experiments show only that a rise of pressure undoubtedly exists in the pulmonary circulation, it might be assumed that it is due solely to back pressure from the left auricle. There is, however, good evidence to show that adrenalin contracts the pulmonary blood vessels, and without entering into the question of pulmonary vasomotors, the following observers, who have noted a rise of pressure in the pulmonary artery or a constriction of the pulmonary vessels after adrenalin, may be mentioned: Weber, E., *Arch. Physiol.*, 1910, Suppl., 410; 1912, 383; 1914, 535. Fühner, H., and Starling, E. H., *J. Physiol.*, 1913-14, xlvii, 301. Tribe, E. M., *ibid.*, 1914, xlviii, 154.

¹⁰ Miller, J. L., and Matthews, S. A., *J. Physiol.*, 1909, iv, 370.

and Matthews state that they used a "considerable number." But artificial respiration was necessary in their experiments to record the pressure in the pulmonary artery, and in view of the statements already made regarding the action of this procedure on the production of pulmonary edema, their failure to obtain edema is readily explained. Hallion and Nepper¹¹ also give as an impression that acute pulmonary edema in rabbits after the intravenous injection of large doses of adrenalin occurred apparently less readily, when the thorax was open than when the chest was intact. They are inclined to attribute this action to mechanical conditions of the experiment, circulatory changes in the lung, and they also mention as a factor the positive intraalveolar pressure produced by artificial respiration.

The question now arises how artificial respiration reduces or prevents the pulmonary edema called forth by adrenalin in rabbits.

Artificial respiration does not prevent cardiac changes resulting in an acute congestion of the lungs, for we have observed, as described previously, such cardiac changes in rabbits when the chest was open. Emerson's tentative explanation¹² that the distension of the lung by artificial respiration drives a considerable amount of blood into the left auricle thus relieving the pulmonary congestion, is unsatisfactory even for the conditions theoretically deduced or experimentally observed by him, for he states that the adrenalin causes acute dilatation of the left ventricle with consequent mitral regurgitation, acute congestion of the lungs, and a dilatation and failure of the right heart; edema results due to the back pressure from the left ventricle. On the basis of this conception it is impossible for us to see how the massage of blood by artificial respiration from the lung into an acutely dilated left ventricle with incompetency of the mitral valves, could reduce the back pressure which, according to Emerson, causes the edema. In this connection we may say that we did not observe any acute dilatation of the left ventricle or regurgitation into the left auricle, nor have we seen any marked dilatation of the right ventricle during the cycle of cardiac changes induced by adrenalin. The left ventricle in the rabbit after adrenalin always seemed in a state of greater tone than normal. A few times the entire heart apparently stopped for a few beats in diastole but without any resulting marked dilatation. We are inclined therefore to believe that

¹¹ Hallion and Nepper, *J. physiol. et path. gén.*, 1911, xiii, 893.

¹² Emerson,¹ p. 370.

artificial respiration with positive pressure does not exert its inhibitory action on pulmonary edema through an action on the heart itself.

There is a factor, however, in the production of pulmonary edema, which is abolished by ordinary artificial respiration, that has received practically no consideration so far as we know. That factor is the aspirating action of the lung alveoli under certain conditions during inspiration. When the diaphragm descends, the negative pressure in the thorax increases, and air enters freely into the alveoli through the trachea and bronchi. The air pressure in the alveoli and consequently upon the capillaries in the alveolar walls remains at atmospheric level. If, however, there is a partial or even complete obstruction in the bronchioles, for example, by contraction of the bronchial muscles, hindering or preventing the entrance of air, then during each inspiration every alveolus connected with such a constricted bronchiole must act like a miniature dry cup, for the pressure in the alveoli and on the capillaries in their walls must decrease as these elastic chambers expand due to the disproportion between intra-alveolar and intrathoracic pressures. If in addition there is a congestion in the pulmonary circulation the passage of a transudate into the alveoli is surely facilitated if not even initiated by this pressure decrease in the alveoli. Experimental support for this view is given by the series of experiments where adrenalin was injected in rabbits whose tracheæ were stenosed.

This conception that the lung alveoli may act like dry cups, has been mentioned as early as 1845 by Mendelsohn.¹³ It must be added, however, that this action of the pulmonary air cells occupies but a subsidiary part in Mendelsohn's elaborate development of the thesis that lung hyperemia in general depends upon diminished expansion and contraction of the lungs during respiration. For, according to Mendelsohn, distension of the lungs stretches the pulmonary artery and its capillaries, increasing their volume capacity and thus aspirating blood; any diminution of the expansion and contraction diminishes this aspiration and reduces the pulmonary circulation which now depends solely upon the propulsive power of the right ventricle, so that stasis occurs.¹⁴

¹³ Mendelsohn, *Arch. physiol. Heilk.*, 1845, iv, 277.

¹⁴ See also Mendelsohn's final publication, *Der Mechanismus der Respiration und Cirkulation, oder das explicierte Wesen der Lungenhyperämien*, Berlin, 1845.

Is there any evidence that such a constriction of the bronchioles occurs after the injection of adrenalin? Since the publication of Kaplan's¹⁵ observation that adrenalin relieves bronchial asthma, most of the investigators describe a relaxation of the bronchial muscles as the result of an adrenalin injection,¹⁶ yet bronchial constriction by the same substance has also been noted. Golla and Symes¹⁷ with a new method of artificial respiration obtained constriction of the bronchioles in decerebrate cats and rabbits after adrenalin unless the bronchioles were initially constricted by other drugs, under which condition a relaxation was obtained. One of us, in some unpublished work, has also observed oncometrically a definite decrease in the amplitude of the lungs in rabbits after the intravenous injection of adrenalin, and it has already been stated that we have often seen a distension of the excised rabbit lung after adrenalin which was not accounted for by the degree of pulmonary edema present.

There is thus sufficient experimental proof that adrenalin may cause a constriction of the bronchial muscles and it is evident that as soon as this occurs the cupping action of the alveoli during inspiration can take place, thus aiding the passage of fluid from the gorged capillaries into the alveoli.

On the basis of this action of adrenalin, the protective action of artificial respiration may be explained; the positive pressure which artificial respiration produces in the lung reduces or overcomes the bronchial constriction due to the adrenalin and thus prevents the intraalveolar pressure from becoming negative during inspiration. Consequently the cupping action on the pulmonary capillaries does not occur. Thus one link in the chain of conditions which are more or less necessary for the production of pulmonary edema is broken, and edema is then produced with greater difficulty and to a less extent.

The protective action of atropine against pulmonary edema from adrenalin after vagus section may also be explained by paralysis of the bronchomotor endings of the vagus, and the consequent inability

¹⁵ Kaplan, D. M., *Med. News*, 1905, lxxxvi, 871.

¹⁶ Januschke, H., and Pollak, L., *Arch. exp. Path. u. Pharm.*, 1911, lxvi, 206-214. Trendelenburg, P., *ibid.*, 1912, lxix, 104. Jackson, D. E., *J. Pharm. and Exp. Therap.*, 1912-13, iv, 74, 291; 1913-14, v, 509. Baehr, G., and Pick, E. P., *Arch. exp. Path. u. Pharm.*, 1913, lxxiv, 62, 71. Dixon, W. E., and Ransom, F., *J. Physiol.*, 1912-13, xlv, 413.

¹⁷ Golla, F. L., and Symes, W. L., *J. Pharm. and Exp. Therap.*, 1913-14, v, 88.

of adrenalin to bring about constriction of the bronchioles, thus preventing the aspirating action of the alveoli. This is in accord with the prevailing view concerning the point of attack of both atropine and adrenalin. It must be mentioned, however, that Golla and Symes¹⁸ report that 10 mg. of atropine in a rabbit failed to relieve the bronchoconstriction caused by adrenalin. It is of course obvious that atropine may also affect the heart or the lung vessels and endothelia in such a way as to prevent or reduce pulmonary edema. But as we have no direct experimental evidence of our own we shall not enter into a discussion of these possibilities.

The aspirating action of the lung alveoli during coexisting pulmonary congestion also explains why the edema is always greater in the lower lobes than in the middle and upper lobes. This is due to the fact that the negative pressure is greater in the lower lobes than in the others. Experimental evidence for this is furnished by the work of Meltzer¹⁹ and Meltzer and Auer,²⁰ which indicates that the negative pressure in the chest is not the same at all levels but is greatest in the lower portion of the thoracic cavity. If a bronchiolar constriction occurs during a marked hyperemia of the lungs it follows that the cupping action of the alveoli during inspiration must be most effective where the negative pressure variation is greatest, provided that the hyperemia and endothelial permeability are the same throughout the lung.

We come now to a consideration of the remarkable accelerating effect which vagus section exerts upon the production of adrenalin pulmonary edema, and it may be stated at once that we cannot offer a full analysis because the experimental test of the various possibilities is incomplete. It may be said, however, that this difference in action is apparently a quantitative one, for pulmonary edema can usually be obtained in the normal rabbit provided that sufficient adrenalin is administered without causing cardiac death. The optimum state for the occurrence of a strong pulmonary edema develops more slowly in a rabbit with vagi intact than in one with vagi sectioned. This suggests that the structure primarily responsible for

¹⁸ Golla and Symes,¹⁷ p. 93.

¹⁹ Meltzer, S. J., *J. Physiol.*, 1892, xiii, 218.

²⁰ Meltzer, S. J., and Auer, J., *J. Exp. Med.*, 1910, xii, 34.

the fulminant onset of edema is the heart. By vagus section the heart is deprived of important regulating influences, and incoordination of the two sides of the heart perhaps then results more readily under stress; that such an incoordination does take place promptly when the vagi are divided and adrenalin is administered, we have already shown. Our experiments on the action of adrenalin on the fully innervated exposed heart of the rabbit are too few to permit any conclusion. The electrocardiographic studies on the action of adrenalin in the dog made by Kahn²¹ and by Rothberger and Winterberg²² which showed no incoordination of the heart after the vagi were sectioned (Kahn), but elicited changes in the form of waves very similar to those obtained by faradic stimulation of the accelerators (Rothberger and Winterberg), tend to support our view because the dog after vagus section tolerates large doses of adrenalin without developing pulmonary edema.

Another action which vagus section may facilitate is the production of bronchial constriction by adrenalin. After vagus section the bronchial muscles are relaxed and thus in the optimum state for contraction as demanded by Golla and Symes. The adjuvant action of bronchial constriction in the production of pulmonary edema has already been discussed.

Vagus section not only facilitates the formation of pulmonary edema after adrenalin, but it is an indispensable preliminary for obtaining pulmonary edema in experimental hydremic plethora.

Kraus²³ reports that the infusion of large quantities of salt solution in cats and rabbits only caused pulmonary edema when the vagi were sectioned. He also observed that adrenalin aided in the production of this edema; that open or closed thorax exerted no influence; and that atropine did not prevent this form of edema. Kraus assumes tentatively that section of centripetal vagus fibers, which govern reflexly the lung vasomotors, causes a disturbance in the regulation of the blood supply to the lung leading indirectly to edema.

Although Kraus' experiments and our own were carried out under widely different conditions so that a comparison cannot be made,

²¹ Kahn, R. H., *Arch. ges. Physiol.*, 1909, cxxix, 379.

²² Rothberger, J., and Winterberg, H., *Arch. ges. Physiol.*, 1910, cxxv, 531.

²³ Kraus, F., *Z. exp. Path. u. Therap.*, 1913, xiv, 402.

it is nevertheless highly suggestive to note than in both sets of experiments vagus section exerted a profound influence. The nature of this influence cannot be stated with certainty at the present time.

In the preceding pages we have made no attempt to give a complete presentation of the problem of pulmonary edema but have limited ourselves largely to those facts which follow directly from our experiments. Necessarily, therefore, numbers of factors have been touched only lightly or not at all in the discussion.

SUMMARY.

The intratracheal injection of one moderate dose of adrenalin in rabbits whose vagi are divided produces a marked pulmonary edema in a large percentage of cases. The same dose in normal animals causes only slight effects.

Artificial respiration greatly reduces the production of pulmonary edema in vagotomized rabbits after adrenalin.

As adrenalin can exert a bronchoconstrictor effect, evidence is submitted to show that the aspirating action of the lung alveoli under this condition apparently plays an important part in the production of adrenalin pulmonary edema. On the basis of this mechanism the protective action of artificial respiration is explained.

Stenosis of the trachea facilitates the production of adrenalin pulmonary edema in rabbits whose vagi are intact.

The intratracheal injection of adrenalin in vagotomized rabbits produces a temporary incoordination between the heart ventricles, visible on inspection, so that the left ventricle beats apparently half as fast as the right, causing hyperemia of the lungs and hemorrhages.

Atropine injected intratracheally in vagotomized rabbits exerts a protective action against adrenalin pulmonary edema.

EXPLANATION OF PLATES.

PLATE 14.

FIG. 1. Blood pressure record. Rabbit 3; black female. Weight 1,980 gm. Both vagi were divided. The time is marked in 4 second intervals. The time line is also the zero pressure level. 0.5 cc. of adrenalin (0.25 cc. per kilo) was injected into the tracheal glass cannula. Note the three abrupt drops in the blood pres-

sure with prompt recovery. The low level (25 to 50 mm. of mercury) was maintained for 20 to 60 seconds, and convulsions appeared during this time. The typical foam of pulmonary edema appeared in the trachea after the second fall, and pink fluid during the terminal descent of the blood pressure. The terminal fall was slowed by a partial clot. These abrupt pressure drops are possibly caused by a complete cardiac stoppage such as was observed when the heart was exposed for inspection.

PLATE 15.

Figs. 2 and 3 show the protective effect of atropine. In both rabbits the vagi were divided and both received 0.25 cc. of adrenalin per kilo intratracheally. Rabbit 5, however, was injected with 1.75 mg. of atropine sulfate per kilo intratracheally before the adrenalin was administered.

FIG. 2. Rabbit 4, adrenalin alone. Typical pulmonary edema developed, with death in 18 minutes. The lungs were large, heavy, erect, hemorrhagic, with foam and fluid in the tracheal cannula.

PLATE 16.

FIG. 3. Rabbit 5, which received atropine before the adrenalin, showed no symptoms of pulmonary edema, and was killed after 45 minutes. The lungs collapsed practically normally, but the surfaces were peppered with small hemorrhages. The trachea showed no foam or fluid.

PLATE 15.

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FIG. 3.

(Auer and Gates: Adrenalin pulmonary edema.)

THE FERROUS SULFATE AND AMMONIA METHOD FOR THE REDUCTION OF NITRO TO AMINO COMPOUNDS.

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The reduction of aromatic nitro compounds to their corresponding amino derivatives has been the subject of such exhaustive treatment in the past and so many excellent methods for accomplishing this end have been devised and so thoroughly discussed in the numerous laboratory manuals that it may seem scarcely necessary to reopen the question. But to those who, like ourselves, may be engaged in investigations which require the convenient and rapid preparation of substances on a scale sufficient for further synthetic work, we feel that our experience may be of service.

In the majority of instances in which nitro compounds have been reduced tin or tin salts have perhaps been the most popular reagent. Except in the case of alkali-insoluble substances, or where the hydrochloride of the base does not form tin double salt, the metal must be removed with hydrogen sulfide, a very time-consuming operation. And when the substances are sensitive to acids this method is to be avoided.

Of the other methods available, that of reduction by means of ferrous sulfate and ammonia has been of service in the past in the preparation of such substances as the aminocinnamic acids, aminopropiolic acid and the otherwise inaccessible *o*-aminobenzaldehyde. It has occasionally been employed with success in other isolated cases, but appears on the whole to have been comparatively neglected. Our experience with the method in reducing a great many substances of varying type would seem to warrant its more frequent employment where the properties of the substances permit. It is the object of this paper to call attention to the ease of manipulation, rapidity, and cheapness of the method, and to the good yields which accompany its proper use.

Our attention was first turned to the method in seeking for a convenient means of reducing substituted nitro amides and nitro ureides. Because of the sensitiveness of these substances to acids and fixed alkali the iron sulfate and ammonia method recommended itself as a milder process, the reaction of the boiling solution being but faintly alkaline. The results obtained were gratifying, for in every instance where other methods were unsatisfactory or had failed this method yielded the desired result.

Another reducing reagent which seemed applicable to our problem was ammonium sulfide. This has, of course, frequently been employed in the past, for example, in the preparation of the aminobenzamides and of *m*-aminobenzoylurea. Our experience has shown that both *p*-nitrobenzamide and *p*-nitrobenzoylurea can be conveniently and rapidly reduced by this reagent. In the case of the isomers, however, it was not only necessary in each case to vary the amount of reagent and the time of the reaction, but the yields obtained were also uncertain. On the other hand the iron sulfate and ammonia method proved to be a uniform, rapid, and reliable procedure.

In the preparation of a considerable number of amino acids from the corresponding nitro compounds this procedure was also found to give very satisfactory results.

In the experimental part below, we have selected a number of examples which emphasize the usefulness of the ferrous sulfate and ammonia method, showing it to be, in most cases, a decided improvement over the methods given in the literature for the preparation of these compounds. Numerous additional instances of the successful use of the method will appear in the communications to follow.

EXPERIMENTAL.

As far as the manipulation is concerned, we have nothing to add to the method, the following procedure being essentially that adopted by previous workers: In the case of nitro acids the substance was first dissolved in sufficient dilute ammonia, using heat to aid solution, if necessary, while in the case of insoluble substances a thin paste was prepared with hot water. The solution or suspension was then poured in a thin stream, with vigorous shaking, into a boiling solution

of 7 mol. equivalents (1 in excess) of ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) in 2–2.5 parts of water. The solution was then immediately treated with small portions of concentrated aqueous ammonia, each addition being followed by vigorous shaking. The addition of ammonia was continued until the boiling solution became definitely alkaline to litmus. The mixture was then boiled 5 minutes and filtered hot with the aid of suction after adding more ammonia if the reaction did not remain alkaline. In many cases the amino compounds separated on cooling; in the case of acids or soluble substances the solution was concentrated to small bulk *in vacuo* or, in the case of amines showing little tendency to oxidize, on the water bath. Amino acids were then liberated from the concentrated solutions of the ammonium salts by the addition of acetic acid. Deviations from the above procedure are mentioned below as they occur.

1. Reduction of Nitro Acids.

m-Aminophenylacetic Acid, $m\text{-H}_2\text{NC}_6\text{H}_4\text{CH}_2\text{CO}_2\text{H}$.—The reduced ammoniacal solution from 39 g. *m*-nitrophenylacetic acid was concentrated on the water bath, filtered, and acidified with acetic acid. 25 g. of the acid separated, melting at 151° . Gabriel and Borgmann,¹ who reduced the nitro acid with tin and hydrochloric acid, give $148\text{--}9^\circ$ as the melting point.

p-Aminophenylacetic Acid.—This acid was prepared exactly as in the case of its isomer. 130 g. of the nitro acid yielded 83 g. of the amino acid, corresponding in properties to those recorded in the literature.

p-Aminophenoxyacetic Acid, $p\text{-H}_2\text{NC}_6\text{H}_4\text{OCH}_2\text{CO}_2\text{H}$.—The *p*-nitrophenoxyacetic acid used as starting material was prepared as follows: 139 g. *p*-nitrophenol, 160 g. 50% sodium hydroxide solution (2 mols.), 95 g. chloroacetic acid (1 mol.), and 800 cc. water were boiled in an open flask until neutral to litmus, about 2 hours being required. Half the above quantities of alkali and chloroacetic acid and 200 cc. water were then added and the solution boiled again until neutral. After acidifying strongly with hydrochloric acid the solution was cooled, precipitating the nitro acid. This was recrystallized from alcohol or puri-

¹ Gabriel and Borgmann, *Ber.*, 16, 2065 (1883).

fied by dissolving in dilute alkali and reprecipitating with hydrochloric acid. The yield was 100–120 g., melting at 183° as recorded in the literature.

The solution obtained by reducing 20 g. of the nitro acid with ferrous sulfate and ammonia was concentrated *in vacuo* until the ammonium salt of the amino acid was salted out by the action of the ammonium sulfate present. The mixture was then heated until solution took place and acetic acid added in excess, the *p*-aminophenoxyacetic acid precipitating out almost immediately. The yield was 15.7 g. According to statements in the literature¹ the acid does not melt below 300°, but all of our samples, whether air-dry or anhydrous, or prepared as above or by hydrolysis of *p*-acetaminophenoxyacetic acid, melted with gas evolution and resolidification at about 220°, the residue not melting below 285°.

Kjeldahl: 0.2204 g. subst.; 17.9 cc. *N*/14 HCl.

Calcd. for $C_8H_9O_3N$: N, 8.38%. Found: N, 8.12%.

2. Reduction of Nitro Amides.

o-Aminobenzamide, $o\text{-H}_2\text{NC}_6\text{H}_4\text{CONH}_2$.—55 g. *o*-nitrobenzamide were suspended in 650 cc. hot water and a solution of 650 g. ferrous sulfate in 1200 cc. hot water added. The mixture was made ammoniacal, with vigorous shaking, boiled 5 minutes, filtered, and evaporated to small bulk on the water bath. The *o*-aminobenzamide was filtered off after cooling, washed with cold water, and dried. Yield, 33 g. leaflets, melting at 109–11.5° with preliminary softening. Previously recorded melting points range from 108° to 113°.

m-Aminobenzamide.—80 g. of powdered *m*-nitrobenzamide were added to a boiling solution of 950 g. ferrous sulfate in 3 liters of water. When all was dissolved, the solution was made ammoniacal, with vigorous shaking, and the boiling continued 5 minutes. The filtrate was then concentrated *in vacuo* until crystallization occurred. The whole was then diluted with a little hot water until clear and allowed to cool in the ice box, the amino amide separating in long, thin, serrated plates melting at 79–80°. The yield was 63 g. As we have been unable to find a description of the anhydrous amide in the lit-

¹ Howard, *Ber.*, 30, 547 (1897); Kym, *J. prakt. Chem.*, [2], 55, 118 (1897).

erature it is given here. The water-free substance is readily obtained from the hydrate by solution in boiling benzene. It separates from the solvent in silky needles which melt at $113-4^{\circ}$ (corr.) and are soluble in ether and difficultly soluble in cold chloroform or benzene.

Kjeldahl: 0.1437 g. subst.; 30 cc. $N/14$ HCl.

Calcd. for $C_7H_8ON_2$: N, 20.59%. Found: N, 20.87%.

3. Reduction of Nitro Ureas.

o-Aminobenzoylurea, $o\text{-H}_2\text{NC}_6\text{H}_4\text{CONHCONH}_2$. — *o*-Nitrobenzoylurea was made by boiling nitrobenzoyl chloride and urea in benzene solution for 8 hours. The product obtained showed the same properties as those recorded by Diels and Wagner,¹ who used no solvent in the preparation. 41 g. of the nitro urea were reduced in 1.3 liters of boiling water with 375 g. ferrous sulfate and an excess of ammonia. An equal volume of 95% alcohol was added to the hot mixture, which was then digested on the water bath for 40 minutes and filtered. The aminobenzoylurea separated on cooling in a yield of 26 g. Recrystallized from 50% alcohol, it forms faintly yellowish leaflets which become pasty and evolve gas at about 200° and immediately resolidify to a product which does not melt below 280° . Diels and Wagner,² who reduced the nitro urea with the aid of zinc dust, describe *o*-aminobenzoylurea as forming brown needles. Contrary to these authors, it may be recrystallized unchanged from boiling acetic acid, as was shown by analysis of the product so obtained, conversion to benzoyleneurea taking place comparatively slowly. In other respects our specimen showed the properties recorded by Diels and Wagner.

0.0995 g. subst.; 19.8 cc. N, 778 mm., 21.0° .

Calcd. for $C_8H_8O_2N_2$: N, 23.46%. Found: N, 23.66%.

m-Aminobenzoylurea.—The necessary *m*-nitrobenzoylurea was prepared by boiling the acid chloride and urea in benzene for 12 hours instead of following Griess' method³ of fusing the components at 150° . The amino urea was obtained in the same way as the *o*-isomer, except

¹ Diels and Wagner, *Ber.*, 45, 880 (1912).

² *Loc. cit.*, p. 881.

³ Griess, *Ber.*, 8, 222 (1875).

that it was necessary to evaporate the alcohol before the substance separated completely on cooling. The yield was 6 g. from 10 g. of the nitro compound. As Griess' description of *m*-aminobenzoylurea is not complete, the following is appended: When rapidly heated it melts with gas evolution at about 210°, resolidifying and then remelting at about 275–80°. It is readily diazotized, in contradistinction to the *o*-isomer, yielding a scarlet color with R-salt, and dissolves readily in boiling water or 95% alcohol.

Kjeldahl: 0.0997 g. subst.; 16.50 cc. 0.1 *N* HCl.

Calcd. for $C_8H_8O_2N_2$: N, 23.46%. Found: N, 23.18%.

METHODS FOR THE ACYLATION OF AROMATIC AMINO COMPOUNDS AND UREAS, WITH ESPECIAL REFER- ENCE TO CHLOROACETYLATION.

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The importance of the halogenacetyl compounds has been demonstrated by their frequent use in organic synthesis. The great reactivity of the halogen atom has permitted their use in practically all the reactions in which alkyl halides have been employed. In the work of the authors on the quaternary salts of hexamethylenetetramine¹ one phase of their usefulness in synthetic work in chemotherapy was demonstrated. In continuing our work along similar lines we have had occasion to prepare the chloroacetyl derivatives of a large number of aromatic amino compounds of such widely differing constitution and solubility relationships that the older acylation methods frequently proved inapplicable.

The well-known Schotten-Baumann method has been quite successfully applied in the past to chloroacetylation. In the case of amino compounds soluble in water or alkali the conditions are simple, although, owing to the great reactivity of chloroacetyl chloride with aqueous alkali, a considerable excess of the chloride is usually required to furnish a satisfactory yield. In the case of bases insoluble in water, the use of neutral organic solvents usually proves of service, either in connection with aqueous alkali, or using an additional equivalent of the base to take up the hydrochloric acid set free. In both cases the method fails with insoluble compounds, while the latter modification is open to the objection that the entire quantity of amine cannot be chloroacetylated in a single operation. The use of pyridine, an excellent solvent for many acylations, does not yield good results when

¹ *J. Biol. Chem.*, 20, 685; 21, 103, 145, 403, 455, 465 (1915); *J. Exp. Med.*, 23, 563, 577 (1916).

chloroacetyl chloride is to be used. We therefore feel that the method outlined below is the most generally satisfactory where chloroacetyl derivatives are in question, and it is here presented not only with this view, but also in the belief that its wide applicability will render it of more general service.

The essential principle of the method is the combined use of dilute acetic acid as solvent and sodium acetate as a buffer to remove the hydrochloric acid formed during the reaction. In such a system the amino compound is held in solution by the acetic acid, which is too feebly acidic to inhibit the specific reactivity of the acid chloride for the amino group. And this is far greater than the rate of hydrolysis of the acid chloride to the acid. As a result, the yields obtained are generally excellent. Acetic acid has, of course, frequently been employed as a solvent in acylations, and the use of sodium acetate to take the place of alkali is also on record, but we have been unable to find a reference to the obvious possibility of their combination.

In the majority of instances 50% acetic acid or, more specifically, a mixture of equal volumes of glacial acetic acid and saturated sodium acetate solution, was found to be most generally serviceable as the solvent, and in but few cases was it necessary to make any change, such as the use of higher concentrations, or the addition of acetone. The method is, of course, inapplicable in cases of insolubility in acetic acid, as, for example, the aromatic amino acids, but where solubility permits, it is our belief that the procedure here described is more satisfactory than the hitherto available methods, not only on account of its wide applicability and its simplicity, but also because the process is carried out in a homogeneous aqueous solution from which the acyl compound separates in practically pure form.

The method was found to be equally satisfactory for benzylation with benzoyl chloride and for phenylchloroacetylation with phenylchloroacetyl chloride, and it can undoubtedly be extended to include other acid chlorides.

In passing from the amines to the ureas a different set of conditions must be taken into account. The substituted aromatic ureas, $RC_6H_4NHCONH_2$, are exceptionally difficult to chloroacetylate, not only because of their sparing solubility in practically all solvents, but also on account of the relative difficulty with which the NH_2 group in

the uramino radical reacts with acid chlorides. It has been the usual custom with the simpler and more soluble members of this group to heat them either with the acid chlorides alone or dissolved in a boiling neutral solvent, from which the liberated hydrochloric acid is evolved. For many of the complex ureas these methods were found unsatisfactory, for although partial reactions occurred, it was practically impossible to separate the chloroacetyl compound from the unchanged urea. This difficulty was finally overcome by the use of chloroacetic acid as a solvent.

The well powdered urea was either dissolved or suspended in molten chloroacetic acid and treated with chloroacetyl chloride. In every case the reaction proceeded smoothly and completely, resulting in a pure product. The use of glacial acetic acid as a substitute for the chloroacetic acid is inadvisable owing to the danger of obtaining acetyl derivatives.

In the experimental part we have not attempted to describe all of the instances in which the above methods were employed, but have selected from our material certain examples to demonstrate their usefulness. A description of other chloroacetyl compounds will appear in later communications.

EXPERIMENTAL.

1. *Chloroacetylation of Amines.*

Unless otherwise stated, the following method was used for chloroacetylating the amino compounds mentioned below. The substance was dissolved in a mixture of five parts of glacial acetic acid and five parts of a saturated solution of sodium acetate, calculating cubic centimeters per gram of substance used. The mixture was warmed, if necessary, to effect complete solution, rapidly chilled in ice-water, and treated with small quantities at a time of one and one-half molecular equivalents of the acid chloride, with vigorous shaking or turbinizing and continuing cooling. The acyl derivative generally separated immediately, and was filtered off after short standing in the cold, washed with 50% acetic acid and water, and recrystallized from the appropriate solvent. The analyses were made and melting points taken after drying to constant weight *in vacuo*, at 100° over concentrated sulfuric acid wherever possible.

Chloroacetanilide.—The crude product obtained from 5 g. aniline was recrystallized from 50% alcohol, yielding 7.75 g. long, narrow, glistening plates which melted at 136–7°, in agreement with the description given in the literature.

Kjeldahl: 0.1601 g. subst.; 9.4 cc. 0.1 N HCl.

Calc. for C_8H_8ONCl : N, 8.26%. Found: N, 8.22%.

p-Iodochloroacetylaniline, *p-IC₈H₄NHCOCH₂Cl*.—An equal weight of recrystallized product was obtained. It separates from 95% alcohol as rods, many of which are branched and curved, melting at 191–4°, the melting point being unchanged by a subsequent recrystallization. The substance is soluble in cold acetone, hot toluene, hot 95% alcohol, and readily soluble in hot glacial acetic acid.

Kjeldahl: 0.2046 g. subst.; 7.0 cc. 0.1 N HCl.

Calc. for C_8H_7ONClI : N, 4.74%. Found: 4.79%.

The compound was also prepared, though less advantageously, by the Schotten-Baumann method.

m-Chloroacetylaminophenol.¹—The reaction mixture was evaporated to dryness *in vacuo* and taken up with water. 22 g. *m*-aminophenol gave 33 g. of the chloroacetyl derivative, melting at 136–8° and agreeing, when recrystallized, with the other properties previously described. The yield far exceeds that obtained by the use of chloroacetic anhydride.

p-Chloroacetylaminophenol.—The reaction mixture from 5 g. of commercial *p*-aminophenol was evaporated to dryness *in vacuo* and the residue taken up with water, filtered, washed with a little water, and recrystallized from 50% alcohol, using boneblack. The yield was 5.5 g. The Schotten-Baumann method gave a smaller yield. The substance may also be recrystallized from water, benzene, or chloroform, and forms rosetts of glistening platelets which melt at 144.5–6° (corr.) with preliminary softening. An aqueous suspension gives a pale grayish color with ferric chloride.

Kjeldahl: 0.2001 g. subst.; 10.80 cc. 0.1 N HCl.

Calc. for $C_8H_8O_2NCl$: N, 7.55%. Found: N, 7.56%.

o-Chloroacetylaminobenzamide.—Addition of chloroacetyl chloride to the solution containing 5 g. *o*-aminobenzamide (see preceding paper)

¹ Jacobs and Heidelberger, *J. Biol. Chem.*, 21, 132 (1915).

produced so thick a mass of the chloroacetyl derivative that it was found necessary to add an additional 50 cc. of 50% acetic acid before the rest of the chloride could be added. 6.5 g. of the product were obtained. Recrystallized from 95% alcohol, the substance forms silky hairs which melt at 183–4.5° with preliminary softening when rapidly heated to 180° and then slowly. It is soluble in acetone, less so in hot chloroform, and difficultly in hot benzene.

Kjeldahl: 0.2808 g. subst.; 26.2 cc. 0.1 *N* HCl.

Calc. for $C_9H_9O_2N_2Cl$: N, 13.18%. Found: N, 13.08%.

The compound was also prepared, though in smaller yield, by dissolving 15.5 g. *o*-aminobenzamide in acetone and adding 10 cc. chloroacetyl chloride, with chilling and shaking, followed by the immediate addition to the thick mass of 50 cc. 10% sodium hydroxide solution, with continued shaking. The mixture was then diluted with water, acidified to congo red with hydrochloric acid and filtered. The yield was 16 g.

m-Chloroacetylaminobenzamide.—In order to dissolve 5.5 g. *m*-aminobenzamide (anhydrous, see preceding paper) completely it was necessary to add an additional 50 cc. of 50% acetic acid to the original mixture of 25 cc. acetic acid and 25 cc. saturated sodium acetate solution. The yield of chloroacetyl derivative was 8 g. Recrystallized from 95% alcohol, the amide forms aggregates of minute crystals which melt with decomposition at about 215°. It is very difficultly soluble in water, benzene, acetone, chloroform, and cold 95% alcohol.

Kjeldahl: 0.2062 g. subst.; 19.35 cc. 0.1 *N* HCl.

Calc. for $C_9H_9O_2N_2Cl$: N, 13.18%. Found N, 13.14%

The compound was also synthesized from *m*-aminobenzoic acid through the chloroacetyl derivative described in the next paragraph. This was converted into the acid chloride by the action of phosphorus pentachloride in benzene suspension on the water bath. Crystallization of the chloride was completed by the addition of ligroin, after which the product was filtered off, washed with ligroin, dissolved in chloroform, and converted into *m*-chloroacetylaminobenzamide by shaking the solution with concentrated ammonia.

m-Chloroacetylaminobenzoic Acid.—14 g. *m*-aminobenzoic acid were suspended in 100 cc. benzene and heated 12 hours under a reflux con-

denser with 12 g. chloroacetyl chloride. The amino acid was gradually replaced by its chloroacetyl derivative. After recrystallization from 50% alcohol, 16 g. of the product were obtained. Recrystallized again from acetic acid, it forms aggregates of minute crystals melting with preliminary softening at 230–2° to a brown liquid, with gas evolution. The acid is less difficultly soluble in acetone than in the other usual solvents in the cold, and is soluble in dilute sodium carbonate solution.

Kjeldahl: 0.2573 g. subst.; 11.85 cc. 0.1 *N* HCl.

Calc. for $C_9H_8O_2NCl$: N, 6.56%. Found: N, 6.45%.

Hexamethylenetetraminium Salt of m-Chloroacetylaminobenzamide.—5.4 g. of recrystallized chloroacetyl compound and 3.8 g. hexamethylenetetramine were boiled for one and one-half hours in 250 cc. acetone. The mixture gradually changed to a thick paste of delicate, felted needles. After letting stand another hour in a warm place the salt was filtered off, boiled again with 100 cc. acetone to remove any unchanged chloroacetyl compound present, filtered, and dried. The yield was 4.5 g. When rapidly heated to 165°, then slowly, the salt darkens and sinters, melting at 169–70° to a greenish tar which quickly decomposes with gas evolution and turns orange. It is soluble in cold water, hot absolute alcohol, and practically insoluble in chloroform or acetone.

0.1439 g. subst.; 0.0584 g. AgCl.

Calc. for $C_{11}H_{12}O_2N_6Cl$: Cl, 10.05%. Found: Cl, 10.04%.

p-Chloroacetylaminobenzamide.—3 g. *p*-aminobenzamide (see introduction, preceding paper) required the addition of 30 cc. of 50% acetic acid to the usual mixture of 15 cc. acetic and 15 cc. saturated sodium acetate solution before complete solution took place. The yield of chloroacetyl derivative was 4.5 g. Recrystallized from 95% alcohol, adding a few drops of aqueous ammonia to the hot solution to hold back any acid present and cooling rapidly, the substance forms silky needles. When rapidly heated to 240° and then slowly, it melts and decomposes at 241–3°. It is less sparingly soluble in hot acetic acid than in the other usual solvents.

Kjeldahl: 0.1940 g. subst.; 18.50 cc. 0.1 *N* HCl.

Calc. for $C_9H_8O_2N_2Cl$: N, 13.18%. Found: N, 13.36%.

The compound was also prepared as in the case of the *o*-isomer by the action of chloroacetyl chloride and aqueous sodium hydroxide in acetone, 15 g. of the aminobenzamide yielding only 14 g. of the chloroacetyl derivative.

p-Aminophenylacetamide.—*p*-Aminophenylacetic acid (see preceding paper) was esterified with methyl alcohol and hydrochloric acid gas. 50 g. of the methyl ester hydrochloride were treated in the cold with 150 cc. concentrated ammonia solution and allowed to stand in a stoppered flask with occasional shaking. The oily ester gradually dissolved and the amide crystallized out. The yield was 30 g. Recrystallized from water, using bone-black to remove the slight color, it forms glistening scales, melting at 161–2° (corr.). Purgotti,¹ who prepared the amide by reducing the nitro compound with ammonium sulfide, reports the melting point as 153–4°.

Kjeldahl: 0.1695 g. subst.; 22.65 cc. 0.1 N HCl.

Calc. for $C_8H_{10}ON_2$: N, 18.67%. Found: N, 18.72%.

p-Chloroacetylaminophenylacetamide.—During the chloroacetylation of 20 g. of the amino compound the reaction mixture set to a thick mass. This was diluted with water, and the substance filtered off, washed, and dried. The yield was 24 g. Recrystallized first from 85% alcohol, then acetic acid, it forms thin, rectangular plates melting to a brown liquid at 191–1.5° (corr.), with slight preliminary softening. The compound is also soluble in hot alcohol, readily in hot acetic acid, practically insoluble in hot benzene, and difficultly soluble in cold acetone. It gives a strong halogen test.

Kjeldahl: 0.1439 g. subst.; 17.7 cc. 0.07 N HCl.

Calc. for $C_{10}H_{11}O_2N_2Cl$: N, 12.37%. Found: N, 12.30%.

2. Phenylchloroacetylation.

The acylation method was next applied to the preparation of several phenylchloroacetyl derivatives, which are described below. The method used was the same as in the case of chloroacetyl chloride, except that one to one and one-tenth mols. of phenylchloroacetyl chloride² were used, a larger excess being unnecessary since the chlo-

¹ Purgotti, *Gazz. chim. ital.*, 20, 598 (1890).

² Prepared according to Staudiger and Bereza, *Ber.*, 44, 536 (1911).

ride does not react as readily with water as chloroacetyl chloride does. In every case the yield was practically quantitative.

p-Phenylchloroacetylaminophenylurea, *p*-PhCHClCONHC₆H₄NHCO-NH₂.—15 g. *p*-aminophenylurea (see following paper) were dissolved in a mixture of 75 cc. acetic acid and 75 cc. saturated sodium acetate solution, cooled in ice-water, and treated with 19 g. phenylchloroacetyl chloride, with shaking and cooling. After dilution with water the precipitate was filtered off and washed with water. Recrystallized from 95% alcohol, with bone-blackening, the urea forms minute platelets and needles which, when rapidly heated to 195° and then slowly, melt and effervesce at 200–1°. It gives a strong Beilstein test and dissolves in boiling dilute, sodium hydroxide solution with a yellow color. It is practically insoluble in hot water or hot benzene, but soluble in hot 95% alcohol or acetone.

0.1372 g. subst.; 17.0 cc. N, 737 mm., 23.5°.

Calc. for C₁₈H₁₄O₂N₂Cl: N, 13.84%. Found: N, 13.84%.

m-Phenylchloroacetylaminophenol, *m*-PhCHClCONHC₆H₄OH. — 5.5 g. *m*-aminophenol were dissolved in 55 cc. acetic acid and 55 cc. saturated sodium acetate solution. The mixture remained clear after addition of the phenylchloroacetyl chloride but deposited the acyl derivative after diluting with water and scratching. Recrystallized first from 50% alcohol, then from toluene, it forms aggregate of spindles which melt with preliminary softening and slow effervescence at 157–8°. It gives a strong Beilstein test and dissolves in dilute sodium hydroxide solution with a pale yellow color which changes to a transient purple on boiling. It is difficultly soluble in cold acetic acid, readily in hot, the solution treated with a drop of aqueous sodium nitrite and warmed at 100°, giving a deep red color.

Kjeldahl: 0.2050 g. subst.; 7.7 cc. 0.1 N HCl.

Calc. for C₁₄H₁₁O₂NCl: N, 5.36%. Found: N, 5.26%.

m-Phenylchloroacetylaminobenzamide, *m*-PhCHClCONHC₆H₄CONH₂. — This substance crystallizes from the reaction mixture after dilution. The product obtained from 7.5 g. *m*-aminobenzamide and 11 g. acid chloride was boiled up with about 100 cc. 50% alcohol and filtered off hot in order to remove any of the more soluble impurities that might be present. Recrystallized from acetic acid, it separates as

micro-platelets which are soluble in boiling 95% alcohol, difficultly soluble in acetone, and very sparingly so in boiling water. Rapidly heated to 210° and then slowly, it melts and decomposes at 218° with slight preliminary softening.

Kjeldahl: 0.1468 g. subst.; 10.0 cc. 0.1 N HCl.

Calc. for $C_{14}H_{13}O_2N_2Cl$: N, 9.71%. Found: N, 9.54%.

p-Phenylchloroacetylaminophenylacetamide, *p*-PhCHClCONHC₆H₄CH₂CONH₂.—The precipitation of this substance from the reaction mixture obtained from 6 g. aminophenylacetamide and 8 g. chloride was completed by the addition of an equal volume of water. Recrystallized first from 50%, then from 95% alcohol it formed minute, thin plates and needles melting at about 184.5–5.5° to a yellow liquid, the exact point of fusion depending somewhat on the rate of heating. A solution in hot water evolves ammonia when boiled with a few drops of sodium hydroxide solution and then reacts for chlorine ion. The substance is soluble in acetone, acetic acid, or hot 95% alcohol, and is practically insoluble in chloroform.

Kjeldahl: 0.1400 g. subst.; 9.35 cc. 0.1 N HCl.

Calc. for $C_{14}H_{13}O_2N_2Cl$: N, 9.26%. Found: N, 9.35%.

3. Benzoylation.

For comparison with the hitherto available methods of benzoylation several simple aromatic amines were benzoylated as in the case of the other acylations, using 1.1 mols. of benzoyl chloride. 5 g. each of aniline, α -naphthylamine, and β -naphthylamine gave, respectively, 8.8, 7, and 7.4 g. of the recrystallized benzoyl derivative, the purity of the product being controlled in each case by analysis and melting-point determination.

4. Chloroacetylation of Ureas.

The following method was used for the chloroacetylation of substituted aromatic ureas in a number of instances to be reported in a later paper, two examples being given below: The finely powdered urea was suspended in 3 parts of chloroacetic acid which had been melted on the water bath, after which 1.5 molecular equivalents of chloroacetyl chloride were added and the heating continued 15–30

minutes. Solution occurred rapidly as a rule, and after completion of the reaction the mixture was poured into water, well stirred, and the resulting precipitate of chloroacetyl derivative washed well with water.

o-Uraminophenyl Benzoate (o-Benzoyloxyphenylurea), $o\text{-H}_2\text{NCONH-C}_6\text{H}_4\text{OCOC}_6\text{H}_5$.—20 g. *o*-uraminophenol were dissolved in 100 cc. pyridine, cooled in a freezing mixture, and carefully treated with 19 g. benzoyl chloride. After standing for one-half hour, the mixture was stirred into an excess of cold, dilute sulfuric acid and the precipitate filtered off, washed with water, and recrystallized from 50% alcohol. The yield was 27 g. Recrystallized again from 95% alcohol, the substance forms aggregates of minute spears which melt slowly at $178\text{--}9^\circ$ (corr.). It is soluble in cold acetone, very difficultly so in boiling water, and almost insoluble in boiling benzene.

0.1481 g. subst.; 13.7 cc. N, 765 mm., 19.5° .

Calc. for $\text{C}_{14}\text{H}_{11}\text{O}_3\text{N}_2$: N, 10.93%. Found: N, 10.87%.

o-Chloroacetyluraminophenyl Benzoate, $o\text{-ClCH}_2\text{CONHCONHC}_6\text{H}_4\text{OCOC}_6\text{H}_5$.—The reaction mixture was heated for only 15 minutes in this case. Recrystallized from acetic acid the benzoate forms voluminous rosetts of silky hairs which readily become triboelectric and which are less sparingly soluble in acetone and ethyl acetate than in the other usual solvents in the cold. Rapidly heated to 215° and then slowly, it melts at 219° with gas evolution and slight preliminary softening. It gives a strong Beilstein test.

0.1315 g. subst.; 9.6 cc. N, 771 mm., 23.0° .

Calc. for $\text{C}_{15}\text{H}_{10}\text{O}_4\text{N}_2\text{Cl}$: N, 8.43%. Found: N, 8.54%.

m-Uraminophenyl Benzoate.—This substance was prepared in essentially the same way as the *o*-isomer. The yield obtained from 29 g. *m*-uraminophenol was 49 g., melting at $178\text{--}80^\circ$. Recrystallized twice from 95% alcohol, using bone-black, it forms lenticular plates which dissolve in acetone, boiling water, and very readily in boiling 95% alcohol. Rapidly heated to 175° , then slowly, the benzoate softens above 180° and melts slowly at $183\text{--}4^\circ$ (corr.), a higher figure being obtained if the final heating is not slow.

0.1590 g. subst.; 14.8 cc. N, 767 mm., 22.5° .

Calc. for $\text{C}_{14}\text{H}_{11}\text{O}_3\text{N}_2$: N, 10.93%. Found: N, 10.85%.

m-Chloroacetyluraminophenyl Benzoate.—After 30 minutes' heating the reaction mixture was poured into ice-water, precipitating the substance as a gum which rapidly crystallized. 15 g. of the urea yielded 18 g. of crude product. Recrystallized first from hot acetone by the addition of an equal volume of water, then from 95% alcohol, the benzoate separates in rosets of long, flat needles which dissolve more readily in boiling acetic acid than in the other usual solvents. Rapidly heated to 185° and then slowly, it softens slightly and melts at 188–9.5° to a brown liquid, with slight gas evolution.

0.1591 g. subst.; 11.7 cc. N, 746 mm., 22.5°.

Calc. for $C_{16}H_{13}O_4N_4Cl$: N, 8.43%. Found: N, 8.34%.

UNSYMMETRICAL DERIVATIVES OF AROMATIC DIAMINES.

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In the course of our synthetic work it was found necessary to prepare a number of derivatives of *m*-phenylenediamine, *p*-phenylenediamine, and 2,4-tolylenediamine. It was soon found that, although a great many of the simpler unsymmetrical derivatives of these amines had been made, notably by Schiff and Vanni¹ and Schiff and Ostrogovich,² previous workers had been unable to isolate all of these substances in a state of purity or else had recorded properties which we were unable to confirm. For example, Schiff and Ostrogovich state that *p*-aminophenylurea is an easily oxidizable substance melting at 129°, whereas all our preparations of this compound consisted of stable crystals melting at 162-4°. Other cases of similar discrepancies will be found in the experimental part. In a number of instances substances had been prepared but never described, as in the case of *m*-aminophenyglycine, while in other instances, for example *m*-aminoxanilic acid, we were unable to find satisfactory directions for making substances which have frequently been used in both research and technical work. We have attempted, therefore, to supply some of the missing material, much of which we believe to be of general interest owing to the fundamental nature of the substances involved. A number of new derivatives of the diamines are also described.

EXPERIMENTAL.

1. Derivatives of *m*-Phenylenediamine.

m-Aminoacetanilide Hydrochloride.—Wallach and Schulze³ describe this substance as melting at about 280°, while Schiff and Ostrogovich⁴

¹ Schiff and Vanni, *Ann.*, 268, 305 (1892).

² Schiff and Ostrogovich, *Ibid.*, 293, 371 (1896).

³ Wallach and Schulze, *Ber.*, 15, 3020 (1882).

⁴ *Loc. cit.*, p. 382.

give 194° as the softening point. By the method described by the former authors 324 g. commercial *m*-phenylenediamine yielded 178 g. of the salt after recrystallizing by bone-black in the minimum amount of hot water, adding an equal volume of 1:1 hydrochloric acid, and chilling. Recrystallized again from 95% alcohol the hydrochloride forms transparent plates which melt at 248–51° with preliminary softening to a thick, turbid, yellow liquid which darkens and becomes entirely fluid at about 280°. It is readily soluble in cold, dry methyl alcohol, less readily in cold water or absolute alcohol, and is readily diazotized, yielding a scarlet color with R-salt.

0.1613 g. subst.; 0.1246 g. AgCl.

Calc. for $C_8H_{10}ON_2.HCl$: Cl, 19.00%. Found: Cl, 19.11%.

m-Aminoacetanilide.—The above authors did not isolate this substance in the pure state, while C. Mills and Lawson¹ obtained it by reduction of *m*-nitroacetanilide, describing it as melting at 87–9°. The base is readily obtained by suspending the hydrochloride in a small volume of water and adding an excess of concentrated sodium hydroxide solution or concentrated aqueous ammonia. It precipitates as an oil which solidifies on rubbing at 0°. The liquid is decanted and the base pressed out on a porous plate, dried *in vacuo*, and recrystallized twice from benzene, when it forms feathery aggregates of flat needles or long, thin plates which melt at 86.5–87.5° (corr.). It is very readily soluble in the cold in water, acetone, and alcohol, less so in chloroform, and difficultly in benzene.

0.1009 g. subst.; 16.4 cc. N, 760 mm., 24°.

Calc. for $C_8H_{10}ON_2$: N, 18.67%. Found: N, 18.68%.

m-Chloroacetylaminacetanilide.—10 g. *m*-aminoacetanilide hydrochloride were chloroacetylated in 50% acetic acid in the presence of sodium acetate, according to the method given in the preceding paper. The chloroacetyl derivative separated as an oil which crystallized almost immediately. The yield was 10.1 g. Recrystallized from 95% alcohol the substance forms radiating masses of delicate needles which melt at 212–4° with slow decomposition and preliminary softening. It dissolves in boiling water, more easily in boiling 95% alcohol,

¹ C. Mills and Lawson, *J. Chem. Soc.*, 67, 927 (1895).

and readily in boiling acetic acid, while it is almost insoluble in hot chloroform. Boiled with dilute sodium hydroxide solution it splits off chlorine ion.

0.1245 g. subst.; 13.2 cc. N, 760 mm., 21.5°

Calc. for $C_{10}H_{11}O_2N_2Cl$: N, 12.37%. Found: N, 12.29%.

m-Acetaminophenylurea, $m\text{-CH}_3\text{CONHC}_6\text{H}_4\text{NHCONH}_2$.—Schiff and Ostrogovich¹ describe this substance as crystallizing in reddish needles which melt at 225° and dissolve readily in water. 10 g. *m*-aminoacetanilide hydrochloride were dissolved in 120 cc. water and 2.5 cc. acetic acid added. The solution was cooled with ice and treated with a concentrated solution of 4.5 g. potassium cyanate. The urea separated on scratching in a yield of 8 g. Recrystallized from 95% alcohol it forms colorless aggregates of thin plates which are difficultly soluble in cold water, more easily in cold alcohol or acetic acid, and readily in all these on warming. Rapidly heated to 200°, then slowly, the urea melts at 204–5.5° with gas evolution.

Kjeldahl: 0.1534 g. subst.; 34.1 cc. N/14 HCl.

Calc. for $C_8H_{11}O_2N_2$: N, 21.76%. Found: N, 22.23%.

m-Aminophenylurea Hydrochloride.—According to Schiff and Ostrogovich² this salt forms scales melting at 281–2°. 67 g. *m*-acetaminophenylurea were boiled 15 minutes with 5 parts (375 cc.) of 1:1 hydrochloric acid (brought to a boil before adding) and rapidly chilled, causing precipitation of the amino salt. This was filtered off and washed with acetone. The yield was 48 g. Recrystallized from 85% alcohol, adding a drop of concentrated hydrochloric acid before cooling, it forms feathery aggregates of microscopic spears which are soluble in cold water but difficultly so in cold alcohol. It sinters and softens at 220–30° and gradually melts and decomposes above 275°.

0.1790 g. subst.; 0.1372 g. AgCl.

Calc. for $C_7H_7ON_2.HCl$: Cl, 18.90%. Found: Cl, 18.96%.

m-Aminophenylurea.—The authors quoted above were unable to isolate the free base from their hydrochloride, although this presented no difficulty in the case of our samples of the salt. A portion was suspended in a small volume of water and an excess of concentrated

¹ *Loc. cit.*, p. 383.

² *Ibid.*, p. 384.

aqueous ammonia added. The base separated from the clear solution on scratching. It was filtered off, washed with a little ice-water, and recrystallized from water, forming radiating groups of long, prismatic needles. The urea melts at 128–30° (corr.) with slight, slow gas evolution and slight preliminary softening, and dissolves very much more easily in hot water or alcohol than in the cold. It is almost insoluble in hot benzene or chloroform, but dissolves in acetone. It is readily diazotized, giving a red color with R-salt.

0.0956 g. subst.; 22.8 cc. N, 759 mm., 18.5°.

Calc. for $C_7H_8ON_2$: N, 27.81%. Found: N, 27.89%.

m-Chloroacetylaminophenylurea, $m\text{-ClCH}_2\text{CONHC}_6\text{H}_4\text{NHCONH}_2$.—20 g. *m*-aminophenylurea hydrochloride, chloroacetylated by the dilute acetic acid method,¹ gave 19 g. of the acyl derivative. Recrystallized first from 50%, then from 95% alcohol, it forms delicate, felted needles which are difficultly soluble in the usual hot, neutral solvents with the exception of alcohol. Rapidly heated to 190°, then slowly, it melts at 192–3° with gas evolution. Boiled with dilute sodium hydroxide solution it splits off chlorine ion.

0.1172 g. subst.; 19.0 cc. N, 756 mm., 24.0°.

Calc. for $C_8H_{10}O_2N_2Cl$: N, 18.47%. Found: N, 18.53%.

m-Aminophenylglycine, $m\text{-H}_2\text{NC}_6\text{H}_4\text{NHCH}_2\text{CO}_2\text{H}$.—This substance is mentioned, but not described, in Ger. pat. 96857.² We obtained it in 75% yield by reduction of the *m*-nitro compound by the ferrous sulfate and ammonia method, using the procedure outlined in the first paper of this series. The amino acid is very susceptible to oxidation when dissolved and is therefore difficult to obtain free from color. Recrystallized from water containing a few drops of acetic acid it forms pale brown, wedge-shaped crystals which are difficultly soluble in the usual neutral solvents except hot water. Rapidly heated to 185°, then slowly, it melts at 193–4° with gas evolution. In water it gives a deep brown color with ferric chloride, while in a solution in dilute hydrochloric acid sodium nitrite gives rise to an orange-colored solution which does not couple with R-salt.

Kjeldahl: 0.1345 g. subst.; 15.90 cc. 0.1 N HCl.

Calc. for $C_8H_{10}O_2N_2$: N, 16.87%. Found: N, 16.57%.

¹ See preceding paper.

² Friedländer, *Fortschr. Teerfarben-Fabrik.*, IV, 924.

m-Aminophenylglycine Methyl Ester Dihydrochloride.—60 g. of the glycine were suspended in 500 cc. dry methyl alcohol and dry hydrochloric acid gas was passed in until saturated. The glycine dissolved, after which the ester salt separated. The mixture was cooled with ice, filtered, and the hydrochloride washed with dry acetone. The yield was excellent. Recrystallized by dissolving in dry methyl alcohol, adding dry ether until faintly turbid, and seeding, the salt was obtained as rosets of minute spears which dissolve in the cold in methyl and ethyl alcohols. Rapidly heated to 190° and then slowly, it softens and decomposes at 196–7°. The salt is readily diazotized, giving a red color with R-salt, and gives a slowly developing brown color with ferric chloride in aqueous solution. With sodium carbonate solution it gave the ester as an oil which did not solidify at 0°.

0.1879 g. subst.; 0.2161 g. AgCl.

Calc. for $C_8H_{11}O_2N_2 \cdot 2HCl$: Cl, 28.41%. Found: Cl, 28.46%.

m-Aminophenylglycinamide, $m-H_2NC_6H_4NHCH_2CONH_2$.—The methyl ester dihydrochloride obtained from 60 g. *m*-aminophenylglycine was covered, with cooling, with 350 cc. concentrated aqueous ammonia and occasionally shaken. A clear solution was first obtained, from which the amide soon began to separate. After standing overnight, this was filtered off, washed with 10 cc. ice-water, and recrystallized from 95% alcohol. The yield was 15 g. A further amount was obtained, however, by concentrating the ammoniacal solution. The amide forms rosets of flat needles which soften at 145° and melt at 145.5–6.5° (corr.). It dissolves readily in methyl alcohol, is soluble in cold water, difficultly in cold 95% alcohol, cold acetone, and scarcely in benzene. It is readily diazotized, giving an orange-red color with R-salt.

Kjeldahl: 0.2074 g. subst.; 37.35 cc. 0.1 N HCl.

Calc. for $C_8H_{11}ON_3$: N, 25.45%. Found: N, 25.23%.

m-Aminoaxanilic Acid, $m-H_2NC_6H_4NHCOCO_2H$.—Although this substance has been well known ever since its discovery by Klusemann,¹ as is evidenced by its frequent use by other writers and in chemical patents, we were unable to find recorded a satisfactory method

¹ Klusemann, *Ber.*, 7, 1263 (1874).

for its preparation. Small amounts may be obtained by the modification of Koller's method given below for the preparation of *p*-aminoxanilic acid, but the yield is poor and the method therefore unsuitable for large-scale work. The following procedure was therefore adopted: 100 g. of technical *m*-phenylenediamine were melted and thoroughly mixed with 240 g. of powdered, crystalline oxalic acid. The mixture was heated 1 hour at 115–20° and 2 hours at 140°, remaining solid during the entire period. After cooling, the product was powdered, suspended in about 4 liters of water and treated with aqueous ammonia until completely dissolved. Calcium chloride was then added in excess, the calcium oxalate filtered off, and acetic acid added to the filtrate until strongly acid. The amino acid quickly separated on scratching. The yield was 85 g. As no complete or accurate description of the substance appears to have been given, the following facts are presented: Recrystallized from water, the acid forms radiating masses of delicate needles which contain one molecule of water of crystallization. The anhydrous acid, rapidly heated to 240° and then slowly, turns yellow and finally melts and decomposes at 245°, not 225°, as given by Schiff and Ostrogovich.¹ It is readily diazotized, giving a deep red color with R-salt.

Air-dry: 0.5428 g. subst.; 0.0468 g. loss. H₂O, 8.62%. Calc.: 9.09%.

Anhydrous: Kjeldahl: 0.1885 g. subst.; 21.1 cc. 0.1 N HCl.

Calc. for C₈H₈O₃N₂: N, 15.56%. Found: N, 15.68%.

Attempts were made to convert the acid into the methyl ester by the action of methyl alcohol and hydrochloric acid gas with a view to preparing the amide in this way, but these resulted only in recovery of the acid hydrochloride or, under vigorous treatment, in decomposition. For this reason the amide was made by the steps outlined below.

m-Nitrooxanilamide, *m*-O₂NC₆H₄NHCOCONH₂.—17 g. *m*-nitroxanilic ethyl ester (from 15 g. *m*-nitraniline and 30 g. ethyl oxalate at 160° for 6 hours) were moistened with alcohol and treated with concentrated aqueous ammonia in excess, with vigorous stirring. Formation of the amide began almost immediately, but the mixture was shaken on the machine for 1 hour and then heated on the water

¹ *Loc. cit.*, p. 385.

bath for 15 minutes. The pasty mass was cooled, filtered, and the substance washed with water and alcohol. The yield of crude amide was 13.8 g. Recrystallized from acetic acid it forms aggregates of minute needles which are more soluble in hot acetic acid than in the other usual solvents. Rapidly heated to 265° and then slowly, it sinters and melts at 268–9° with effervescence.

0.1012 g. subst.; 17.4 cc. N, 760 mm., 21.5°.

Calc. for $C_8H_7O_4N_3$: N, 20.09%. Found: N, 19.94%.

m-Aminooxanilamide, *m*- $H_2NC_6H_4NHCOCONH_2$.—26.2 g. of the nitro amide were ground to a thin paste with hot water and reduced by the ferrous sulfate and ammonia method (see the first paper of this series). The aminoamide separated from the ammoniacal filtrate on cooling, the yield being 17.4 g. Recrystallized from water, with bone-blackening, it forms thin, colorless, hexagonal platelets which melt at 191–1.5° (corr.) with slight preliminary softening. The amide is soluble in acetone, hot water, hot 95% alcohol, difficultly in hot chloroform or absolute alcohol, almost insoluble in hot benzene, and separates from the solution in absolute alcohol in woolly masses of delicate needles. It is readily diazotized, yielding a scarlet precipitate with R-salt.

0.1163 g. subst.; 23.75 cc. N, 760 mm., 22.0°.

Calc. for $C_8H_7O_2N_3$: N, 23.46%. Found: N, 23.60%.

m-Chloroacetylaminooxanilamide, *m*- $ClCH_2CONHC_6H_4NHCOCONH_2$.—10 g. of the aminoamide were dissolved in 250 cc. 50% acetic acid containing 15 g. sodium acetate, cooled in ice-water, and treated with 7.5 g. chloroacetyl chloride in small portions, with shaking and cooling. The acyl derivative separated immediately and was filtered off after half an hour's standing and washed with 50% acetic acid and water. The yield was 13 g. A small portion was recrystallized from boiling water, in which it is difficultly soluble, and separated as arborescent masses of minute needles with a faint pinkish tinge. The compound darkens above 270° and decomposes slowly at 281°, the highest temperature reached by the bath. It gives the Beilstein test, and dissolves more easily in boiling acetic acid than in the other usual solvents, in which it is very sparingly soluble.

0.1087 g. subst.; 15.5 cc. N, 765 mm., 20.5°.

Calc. for $C_{10}H_{10}O_2N_3Cl$: N, 16.44%. Found: N, 16.70%.

m-Uraminooxanilamide, $m\text{-H}_2\text{NCONHC}_6\text{H}_4\text{NHCOCONH}_2$.—A solution of 6 g. potassium cyanate was added to a well-chilled solution of 12 g. *m*-aminooxanilamide in 200 cc. 50% acetic acid. The urea separated almost immediately, and was filtered off and washed with water. The yield obtained was 14 g. A small portion was recrystallized from water, with bone-blackening and addition of a few drops of ammonia to the filtered solution. It separates as voluminous, hair-like needles which soften, turn slightly yellow, and decompose somewhat above 260° but do not melt below 280° . The urea is very difficultly soluble in the usual solvents.

Kjeldahl: 0.0645 g. subst.; 11.65 cc. 0.1 N HCl.

Calc. for $\text{C}_8\text{H}_{10}\text{O}_2\text{N}_4$: N, 25.23%. Found: N, 25.30%.

m-Chloroacetyluraminooxanilamide, $m\text{-ClCH}_2\text{CONHCONHC}_6\text{H}_4\text{NHCOCONH}_2$.—6 cc. chloroacetyl chloride were added to a suspension of 11 g. of finely powdered *m*-uraminooxanilamide in 35 g. of molten chloroacetic acid. The urea dissolved on heating on the water bath, except for a few lumps which had to be broken up with a stirring rod before solution would take place. After half an hour's heating the chloroacetyl derivative started to crystallize, whereupon the mixture was diluted with water. The product was filtered off, ground up in a mortar with water, and treated with sodium acetate solution until neutral to congo red. After filtering, washing, and drying, the substance weighed 11.5 g. Recrystallized from acetic acid, in which it is more soluble than in the other usual solvents, it forms minute plates and rosetts of minute, flat needles. Rapidly heated to 230° and then slowly, it decomposes at $233\text{--}4^\circ$ with preliminary darkening and softening. The compound dissolves in boiling, dilute sodium hydroxide solution, splitting off chlorine ion.

Kjeldahl: 0.1428 g. subst.; 19.2 cc. 0.1 N HCl.

Calc. for $\text{C}_{11}\text{H}_{11}\text{O}_4\text{N}_4\text{Cl}$: N, 18.77%. Found: N, 18.83%.

m-Nitromalonanilic Ethyl Ester, $m\text{-O}_2\text{NC}_6\text{H}_4\text{NHCOCH}_2\text{CO}_2\text{C}_2\text{H}_5$.—30 g. *m*-nitraniline and 50 g. malonic ester (1.5 mols.) were heated in an oil bath to 200° , at which point vigorous evolution of alcohol began. The melt was heated one hour at $200\text{--}10^\circ$, allowing most of the alcohol to boil off through an air-condenser, according to the method used by

Chattaway and Mason¹ in obtaining halogen-substituted malonanilic compounds. The excess of malonic ester was then distilled off *in vacuo* and most of the thick, dark residue withdrawn for conversion into the amide. A portion was rubbed with alcohol in a freezing mixture until crystalline and pressed out on a porous plate at 0°. The remaining solid was dissolved in boiling benzene, cooled, filtered from the *m*-nitromalonanilide that separated (see below), and evaporated to dryness. On cooling, the residue crystallized to a mass of long needles. This was dissolved in a little hot benzene and fractionally precipitated by ligroin, discarding the first red, oily fractions and keeping the lighter deposit which subsequently came down and soon crystallized on rubbing. This was recrystallized from ligroin, forming delicate, cream-colored needles which melted at 73.5–4° (corr.) with slight preliminary softening and dissolved readily in the usual solvents except water and ligroin.

0.1387 g. subst.; 13.2 cc. N, 768 mm., 22.5°.

Calc. for $C_{11}H_{13}O_4N_2$: N, 11.11%. Found: N, 11.11%.

m-Nitromalonanilide, $m-O_2NC_6H_4NHCOCH_2CONHC_6H_4NO_2$.—Most of the crude, tarry ester obtained above was thinned with an equal volume of alcohol and shaken on the machine with 2 volumes of concentrated aqueous ammonia. The microcrystalline paste was filtered and the residue washed with water and recrystallized from 95% alcohol, part separating crystalline and part as a jelly which gradually solidified when stirred at frequent intervals. The yield of crude product was 25 g. If any *m*-nitromalonanilamide was present it passed into the mother liquors on recrystallization, for, recrystallized twice from acetic acid and once from amyl alcohol the crude product yielded only *m*-nitromalonanilide in the form of cream-colored needles which melted and resolidified at about 173–7° and finally melted at 198–200° (corr.). Owing to the pressure of other work the more soluble fractions were not investigated. The anilide is soluble in acetone, hot acetic acid, hot amyl alcohol, and almost insoluble in boiling water or benzene.

0.1000 g. subst.; 14.2 cc. N, 759 mm., 24.0°.

Calc. for $C_{18}H_{15}O_6N_4$: N, 16.28%. Found: N, 16.30%.

¹ Chattaway and Mason, *J. Chem. Soc.*, 97, 339 (1910).

m-Aminomalonanilide, $m\text{-H}_2\text{NC}_6\text{H}_4\text{NHCOCH}_2\text{CONHC}_6\text{H}_4\text{NH}_2$.—Most of the crude material containing the nitroanilide was reduced by the ferrous sulfate and ammonia method. A small amount of the aminoanilide separated from the ammoniacal filtrate on cooling. This was purified by suspending in water, adding a slight excess of dilute hydrochloric acid to dissolve the base, adding a little bone-black, and filtering. The base was then precipitated by the addition of ammonia and recrystallized from 50% alcohol. It forms glistening, cream-colored needles which melt at 180.5° (corr.) with slight preliminary softening and are soluble in acetone, boiling water or alcohol, and difficultly in hot benzene. It is readily diazotized, yielding a difficulty soluble scarlet dye with R-salt, and gives a brown color with ferric chloride when dissolved in hot water.

0.1053 g. subst.; 18.0 cc. N, 757 mm., 21.5° .

Calc. for $\text{C}_{14}\text{H}_{16}\text{O}_2\text{N}_4$: N, 19.72%. Found: N, 19.73%.

2. Unsymmetrical Derivatives of *p*-Phenylenediamine.

p-Chloroacetylaminacetanilide, $p\text{-ClCH}_2\text{CONHC}_6\text{H}_4\text{NHCOCH}_3$.—15 g. *p*-aminoacetanilide were dissolved in a mixture of 75 cc. acetic acid and 75 cc. saturated sodium acetate solution and chloroacetylated as in previous examples. The yield was 21 g. Recrystallized from acetic acid the substance forms minute prisms with wedge-shaped ends, which darken above 260° and decompose at $265\text{--}70^\circ$. The compound is very difficultly soluble in the usual solvents.

0.1509 g. subst.; 13.50 cc. 0.1 N HCl. 0.2500 g. subst.; 0.1557 g. AgCl.

Calc. for $\text{C}_{10}\text{H}_{11}\text{O}_2\text{N}_2\text{Cl}$: N, 12.37%; Cl, 15.65%. Found: N, 12.53%; Cl, 15.41%.

p-Acetaminophenylurea.—Owing to the difficulty of obtaining potassium cyanate at the present time the following method of preparation of this urea may be of interest: 100 g. *p*-aminoacetanilide were dissolved in 5 parts of 50% acetic acid, cooled to 0° , turbid, and treated slowly with an aqueous suspension of 65 g. of commercial sodium cyanate. The urea separated on standing and was filtered off and washed with water. The yield was 90 g., corresponding in properties to those given by Schiff and Ostrogovich.¹

¹ *Loc. cit.*, p. 375.

p-Acetaminophenylchloroacetylurea, $p\text{-CH}_3\text{CONHC}_6\text{H}_4\text{NHCONHCO-CH}_2\text{Cl}$.—11 g. *p*-acetaminophenylurea were dissolved in 30 g. chloroacetic acid on the water bath, treated with 5.5 cc. chloroacetylchloride and heated one-half hour on the water bath. The solution was then poured into water and the precipitate filtered off, thoroughly agitated with hot water containing enough sodium acetate to combine with any free hydrochloric acid present, filtered again and washed with water and alcohol. The yield was 11 g. A small portion was recrystallized for analysis from amyl alcohol, forming lenticular microplatelets which are very difficultly soluble in the usual neutral solvents. Rapidly heated to 230° and then slowly, it melts and decomposes at $235\text{--}7^\circ$, with preliminary softening.

0.1278 g. subst.; 17.2 cc. N, 757 mm., 25.0° .

Calc. for $\text{C}_{11}\text{H}_{12}\text{O}_4\text{N}_2\text{Cl}$: N, 15.59%. Found: N, 15.36%.

p-Aminophenylurea Hydrochloride.—This salt, presumably obtained by Schiff and Ostrogovich, was prepared by us as follows: One liter of boiling 1 : 1 hydrochloric acid was poured onto 200 g. *p*-acetaminophenylurea and the mixture boiled 15 minutes. On rapidly chilling the hydrochloride separated at once. This was filtered off, washed with 10% aqueous hydrochloric acid and purified by dissolving in the minimum amount of hot water, adding concentrated hydrochloric acid until the concentration of acid reached about 10%, and chilling rapidly. 100 g. of the salt separated as micaceous plates which did not melt below 280° . Its aqueous solution gives a deep purple color with ferric chloride and is readily diazotized, yielding a purplish red dye with R-salt.

0.1579 g. subst.; 31.0 cc. N, 762 mm., 23.5° .

Calc. for $\text{C}_7\text{H}_8\text{ON}_2\cdot\text{HCl}$: N, 22.40%. Found: N, 22.65%.

p-Aminophenylurea.—When the above hydrochloride is dissolved in a small amount of warm water and treated with an excess of sodium hydroxide or ammonia the free base separates on rubbing as glistening platelets. Schiff and Ostrogovich¹ were unable to isolate the urea in this simple way from their sample of the hydrochloride, and their description of the urea itself is such as to throw doubt on their

¹ *Loc. cit.*, p. 376.

having had the compound in their hands, although Pierron,¹ who claims to have prepared the substance both by reduction of the nitrourea and by hydrolysis of the cyanamide, gives the same melting point as Schiff and Ostrogovich found, namely, 129°. We found that the base, when recrystallized from a small volume of water, with bone-black, forms flat, colorless, glistening needles or long platelets which contain water of crystallization which is only slowly lost on exposure to the air. In one case the compound came to constant weight in the air while still containing one-half molecule of water of crystallization. The urea melts with slight preliminary softening at 162–4° with gas evolution and resolidification, the decomposition product remaining solid below 285°. It is not readily oxidized in the air, as claimed by Schiff and Ostrogovich, and is readily diazotized, coupling with R-salt to yield a purplish red dye. In aqueous solution it gives a brown color with a drop of ferric chloride solution, and deposits purple flocks on addition of a larger amount of the reagent. The urea is soluble in water or acetone, sparingly in cold absolute alcohol, readily in hot, and is almost insoluble in benzene or ether.

Kjeldahl: 0.0963 g. subst.; 26.95 cc. *N*/14 HCl.

Calc. for $C_7H_8ON_2$: N, 27.81%. Found: N, 27.98%.

p-Chloroacetylaminophenylurea, $p\text{-ClCH}_2\text{CONHC}_6\text{H}_4\text{NHCONH}_2$.—38 g. *p*-aminophenylurea hydrochloride were dissolved by warming in a mixture of 150 cc. acetic acid and 150 cc. saturated sodium acetate solution and were then chloroacetylated as in previous examples. After recrystallization from 50% alcohol the yield was 32 g. Recrystallized again from the same solvent, using bone-black, the acyl derivative forms colorless hairs and aggregates of flat needles which are difficultly soluble in the usual neutral solvents. When rapidly heated, the substance melts completely at about 225° with gas evolution and resolidification to a mass which does not melt below 285°, while if the heating is slower, it melts partly at 212–3° with gas evolution and resolidification.

Kjeldahl: 0.1315 g. subst., 17.20 cc. 0.1 *N* HCl.

Calc. for $C_8H_9O_2N_2Cl$: N, 18.47%. Found: N, 18.32%.

¹ Pierron, *Ann. chim. phys.*, [8] 15, 181, 188 (1908).

The glycine derivatives described below were encountered in attempts to obtain good yields of *p*-aminophenylglycine by a convenient method. That involving the reduction of *p*-nitrophenylglycine proved unsuitable because of the poor yields of the nitro compound obtained when the directions given in Ger. pat. 88433¹ were followed. The preparation of the acetyl derivative was then attempted by reacting *p*-aminoacetanilide with chloroacetic acid in aqueous solution, and while this yielded fairly satisfactory results for further synthetic work, the product contained considerable quantities of the diglycine described in the next paragraph, which was the only substance isolated analytically pure, as the soluble *p*-acetaminophenylglycine passed into the mother liquors on recrystallization. Excellent results were finally obtained as described below by the interaction of *p*-aminoacetanilide with chloroacetic ester in alcoholic solution.

p-Acetaminophenyldiglycine, $p\text{-CH}_3\text{CONHC}_6\text{H}_4\text{N}(\text{CH}_2\text{CO}_2\text{H})_2$.—10 g. *p*-aminoacetanilide were suspended in 50 cc. water and boiled until neutral with one molecular equivalent each of chloroacetic acid and sodium hydroxide. Half the amounts of chloroacetic acid and alkali were again added and the mixture boiled until neutral once more. After cooling and filtering, the acetamino acid was precipitated by acidifying the solution with hydrochloric acid until acid to congo red. The crude acid was decolorized by boiling with 85% alcohol containing acetic acid and was then recrystallized from a large volume of water, forming sheaves of almost colorless needles which dissolve in boiling acetic acid with gas evolution. Rapidly heated to 230° and then slowly, the acid darkens slightly above 210° and melts with effervescence at 234–5°.

0.1328 g. subst.; 12.45 cc. N, 763 mm., 22.5°.

Calc. for $\text{C}_{12}\text{H}_{14}\text{O}_6\text{N}_2$: N, 10.55%. Found: N, 10.87%.

p-Aminophenylglycine was finally prepared as follows:

p-Acetaminophenylglycine Ethyl Ester, $p\text{-CH}_3\text{CONHC}_6\text{H}_4\text{NHCH}_2\text{CO}_2\text{C}_2\text{H}_5$.—175 g. *p*-aminoacetanilide were dissolved in 750 cc. hot 50% alcohol and boiled 1.5 hours under a reflux condenser with 76 g. ethyl chloroacetate. On cooling in the ice box the solution set to a solid mass. This was disintegrated, filtered off, and washed thoroughly

¹ Friedländer, *Festschr. Teerfarben-Fabrik.*, IV, 1156.

with water in order to remove the difficultly soluble aminoacetanilide hydrochloride. The yield of crude ester was 100 g. Most of the unused aminoacetanilide can be recovered from the filtrate by neutralization and saturation with salt. Recrystallized first from 95% alcohol, then from benzene, the ester forms rosetts of long, delicate needles which melt at 124–5° (corr.) with slight preliminary softening. It is readily soluble in acetone, chloroform, hot benzene, alcohol, or water, and less readily so in the last three in the cold.

Kjeldahl: 0.1336 g. subst.; 11.25 cc. 0.1 *N* HCl.

Calc. for $C_{12}H_{16}O_4N_2$: N, 11.87%. Found: N, 11.80%.

p-Aminophenylglycine.—104 g. crude *p*-acetaminophenylglycine ethyl ester were boiled one hour under a reflux condenser with 520 cc. 1:1 hydrochloric acid. The solution was cooled and the precipitate of amino acid hydrochloride filtered off, washed with a little 1:1 hydrochloric acid, dissolved in water, and neutralized to congo red with saturated sodium acetate solution. The precipitate of aminophenylglycine was filtered off and washed with water. The yield was 61 g. Recrystallized from water containing a few drops of acetic acid, using bone-black, the glycine was obtained as almost colorless, glistening leaflets which contain one molecule of water of crystallization. On boiling these with absolute alcohol, the crystalline form suddenly changes to microscopic hairs, probably due to dehydration of the hydrate. Air-dry, or anhydrous, when rapidly heated to 220° and then slowly, the compound melts and decomposes at 222–3° with preliminary slight decomposition, not at 208°, as given in Ger. pat. 88433,¹ according to which the aminoglycine is obtained by reduction of the nitroglycine with tin and hydrochloric acid.

0.6162 g. subst.; 0.0611 g. loss. Calc.: 1 H_2O , 9.79%. Found: 9.91%.

0.1145 g. subst.; 16.6 cc. N, 752 mm., 19.5°.

Calc. for $C_8H_{10}O_3N_2$: N, 16.87%. Found: N, 16.74%.

p-Aminophenylglycine Ethyl Ester Dihydrochloride.—7.5 g. of recrystallized *p*-acetaminophenylglycine ethyl ester were dissolved in 40 cc. absolute alcohol which had been saturated at room temperature with dry hydrochloric acid gas. The solution was boiled under a

¹ *Loc. cit.*

reflux condenser for three-quarters of an hour. The crystalline product was filtered off on cooling and washed with a little absolute alcohol. The yield of dihydrochloride was 7.5 g., melting at 201–2° with gas evolution and preliminary softening. The salt is readily soluble in the cold in water or dry methyl alcohol, but rather difficultly in absolute alcohol. In dilute hydrochloric acid it gives a deep, olive-green color with sodium nitrite. A concentrated aqueous solution, treated with sodium carbonate, deposits the ester as an oil.

0.1296 g. subst.; 12.1 cc. N, 764 mm., 22.5°. Carius: 0.1605 g. subst.; 0.1703 g. AgCl. Calc. for $C_{10}H_{14}O_2N_2 \cdot 2HCl$: N, 10.48%; Cl, 26.55%. Found: N, 10.85%; Cl, 26.25%.

p-Aminophenylglycinamide.—108 g. of the ethyl ester dihydrochloride were carefully treated, with chilling, with 3 parts of concentrated aqueous ammonia. The mixture, which darkened and soon began to deposit the amide, was allowed to stand in the cold for 2 days, after which the amide was filtered off, washed with a little alcohol, and recrystallized from 95% alcohol. The yield was 30 g. Again recrystallized from the same solvent, using bone-black, it forms crusts consisting of slightly brownish aggregates of thin plates which melt to a brown liquid at 161–4° with slight preliminary softening. It is soluble in cold water or 95% alcohol, very much more so on heating, and dissolves more readily in acetone than in benzene. In aqueous solution it gives an olive color with ferric chloride, changing to purple. The amide is readily diazotized, the solution giving a red color with R-salt.

0.1036 g. subst.; 23.2 cc. N, 753 mm., 27.0°.

Calc. for $C_8H_{11}ON_3$: N, 25.45%. Found: 25.25%.

p-Aminooxanilic Acid, $p-H_2NC_6H_4NHCOCO_2H$.—This substance was prepared essentially as described by Koller,¹ except that, in order to avoid precipitation of the oxalate of the acid instead of the acid itself, as sometimes happened, it was found advisable to take up the entire reaction mixture in a large volume of water, add aqueous sodium hydroxide solution until alkaline, and precipitate the unchanged oxalic acid with calcium chloride solution. The aminooxanilic acid then separates from the filtrate on adding concentrated hydrochloric acid until the reaction is just faintly blue to congo red paper. The result-

¹ Koller, *Ber.*, 36, 413 (1903).

ing product may be used for further synthetic work without recrystallization.

p-Chloroacetylaminooxanilic Acid, $p\text{-ClCH}_2\text{CONHC}_6\text{H}_4\text{NHCOCO}_2\text{H}$.—Owing to the fact that *p*-aminooxanilic acid is practically insoluble in cold 50% acetic acid it was chloroacetylated by the method usually employed by us for the chloroacetylation of ureas (see preceding paper). 10 g. *p*-aminooxanilic acid were suspended in molten chloroacetic acid and treated with 5 cc. chloroacetyl chloride. Interaction occurred at once, with liberation of hydrochloric acid. After 15 minutes on the water bath the mixture was diluted with water, treated with an excess of hydrochloric acid, and the acyl derivative filtered off and washed with acetone. The yield was 13 g. The substance is extremely difficultly soluble in the usual solvents and forms a sparingly soluble sodium salt when an attempt is made to dissolve it in sodium hydroxide solution. A small portion was therefore purified by suspending in hot water, adding an insufficient amount of ammonia, filtering, and adding hydrochloric acid to the cooled filtrate. The acid precipitated as a gelatinous mass which soon changed to radiating masses of microscopic needles and hairs. Rapidly heated to 230°, then slowly, it darkens slightly above 220° and effervesces at about 235°. It gives a strong Beilstein test and becomes strongly triboelectric when rubbed.

Kjeldahl: 0.1748 g. subst.; 19.3 cc. *N*/14 HCl.

Calc. for $\text{C}_{10}\text{H}_8\text{O}_4\text{N}_2\text{Cl}$: N, 10.92%. Found: N, 11.04%.

p-Aminooxanilic Methyl Ester Hydrochloride.—19.4 g. *p*-aminooxanilic acid were suspended in 200 cc. dry methyl alcohol and turbined, passing in a vigorous stream of dry hydrochloric acid gas through a wide tube to avoid the clogging which otherwise occurs. After a short time the amino acid went into solution, and the ester hydrochloride then separated. This was filtered off, washed with a little dry methyl alcohol, and dried *in vacuo* over calcium chloride and potassium hydroxide. The yield was 22.4 g. Recrystallized from 85% alcohol it forms aggregates of long, thin, slightly purplish plates, which, when rapidly heated, turn yellow above 220° but do not melt below 280°. The salt is soluble in water, and difficultly soluble in hot absolute alcohol or cold, dry methyl alcohol.

0.1417 g. subst.; 0.0858 g. AgCl.

Calc. for $C_9H_{10}O_3N_2 \cdot HCl$: Cl, 15.38%. Found: Cl, 14.98%.

p-Aminooxanilic Methyl Ester.—A supersaturated solution of the hydrochloride was obtained by rapidly cooling a hot, concentrated solution. To this was added a slight excess of 10% sodium carbonate solution, precipitating the ester. This was filtered off, washed with a little cold water, and recrystallized from water, bone-blackening the hot solution. It forms greenish yellow, silky needles which soften at 128° and melt at 129–30° (corr.), resolidifying on cooling. The melting point is unchanged by another recrystallization from benzene. The ester is difficultly soluble in cold benzene, somewhat soluble in cold water, and more readily so in cold 95% alcohol or chloroform. An aqueous suspension gives a slowly-developing reddish brown color with ferric chloride. The ester is also readily diazotized, giving a red dye with R-salt.

Kjeldahl: 0.1423 cc. subst.; 14.45 cc. 0.1 N HCl.

Calc. for $C_9H_{10}O_3N_2$: N, 14.44%. Found: N, 14.23%.

p-Aminooxanilamide, $p-H_2NC_6H_4NHCOCONH_2$.—22 g. powdered *p*-aminooxanilic methyl ester hydrochloride were added in small amounts to a cold, well-stirred, concentrated ammonia solution containing a little alcohol. A few lumps were pressed out with a stirring rod and the pasty mixture was allowed to stand overnight protected from the air. The product was filtered off, washed with a little water, and recrystallized from boiling water, from which it separates as slightly purplish microcrystals which melt at 217–8° to a dark tar. The yield was 10 g. The amide is somewhat soluble in cold water, readily in hot, and dissolves also in acetone, hot 95% alcohol, and boiling ethyl acetate. The aqueous solution gives a slowly-developing purplish color with ferric chloride and, on diazotization and coupling with R-salt, yields a purplish red dye.

Kjeldahl: 0.1537 g. subst.; 25.8 cc. 0.1 N HCl.

Calc. for $C_9H_9O_2N_2$: N, 23.46%. Found: N, 23.52%.

p-Aminooxanilamide was also prepared by the following series of reactions: Oxanilic ethyl ester was dissolved in concentrated sulfuric acid and treated with a solution of 1 mol. of concentrated nitric acid in concentrated sulfuric acid, keeping the temperature below 20°.

After standing for a short time the mixture was poured into ice-water, the *p*-nitrooxanilic ethyl ester crystallizing almost immediately. This was recrystallized from alcohol and then converted into the amide by grinding to a paste with alcohol and adding concentrated aqueous ammonia. After half an hour the product was filtered off and washed with water. A small portion, recrystallized from 95% alcohol gave a good analysis (N, 19.97%; calcd., N, 20.09%) and agreed in all its properties with those described for *p*-nitrooxanilamide by Schultz, Rohde and Herzog.¹ The crude nitroamide, reduced by the ferrous sulfate and ammonia method, yielded the aminoamide, which separated from the ammoniacal filtrate on cooling. A further small quantity could be obtained by again boiling the precipitate of ferric hydroxide with water containing a little ammonia. The amide prepared in this manner was identical in every way with that obtained through the ester, but the yield was not so good, nor was the method as convenient.

p-Chloroacetylaminooxanilamide, $p\text{-ClCH}_2\text{CONHC}_6\text{H}_4\text{NHCOCONH}_2$.—10 g. of the aminoamide were dissolved in 300 cc. 50% acetic acid containing 15 g. sodium acetate and chloroacetylated as in previous examples. The crude product was purified by warming on the water bath with 50% alcohol and filtering hot. The residue weighed 11 g. A small portion was recrystallized from acetic acid, forming sheaves of microcrystals which did not melt below 280° and were difficultly soluble in the usual solvents.

0.1022 g. subst.; 14.5 cc. N, 751 mm., 21.0°.

Calc. for $\text{C}_{10}\text{H}_{10}\text{O}_2\text{N}_2\text{Cl}$: N, 16.44%. Found: N, 16.28%.

p-Nitromalonanilide, $p\text{-O}_2\text{NC}_6\text{H}_4\text{NHCOCH}_2\text{CONHC}_6\text{H}_4\text{NO}_2$.—27.6 g. *p*-nitraniline and 50 g. malonic ester (1.5 mols.) were heated 1 hour at 200–10°, letting the temperature rise to 230° at the end for a few minutes. The excess of malonic ester was distilled off *in vacuo* and the hot residue mixed with about 2 volumes of hot alcohol. The solution was allowed to stand, with occasional rubbing, the anilide gradually crystallizing. This was filtered off, washed with a little alcohol (yield 10 g.), and recrystallized twice from 95% alcohol, forming flat, brownish yellow needles which melted at 241–2° with gas

¹ Schultz, Rohde and Herzog, *J. prakt. Chem.*, [2] 74, 82 (1906).

evolution and slight preliminary softening. The compound is somewhat soluble in cold acetone, readily in hot, and dissolves also in hot acetic acid or alcohol.

0.1136 g. subst.; 15.95 cc. N, 764 mm., 20.0°.

Calc. for $C_{14}H_{13}O_4N_4$: N, 16.28%. Found: N, 16.45%.

p-Nitromalonanilic Ethyl Ester, $p\text{-O}_2\text{NC}_6\text{H}_4\text{NHCOCH}_2\text{CO}_2\text{C}_2\text{H}_5$.—The filtrate from the crude nitromalonanilide was cautiously diluted with water, depositing an oil which gradually crystallized. This was filtered off and dried. The yield was 25 g. Recrystallized from ligroin, in which it is very difficultly soluble, the ester forms pale yellow, hair-like needles or thin, narrow platelets. These melt at 92–5° and are very readily soluble in alcohol or ether, less easily in benzene.

0.1531 g. subst.; 15.4 cc. N, 756 mm., 22.0°.

Calc. for $C_{11}H_{13}O_4N_2$: N, 11.11%. Found: N, 11.58%.

p-Nitromalonanilamide, $p\text{-O}_2\text{NC}_6\text{H}_4\text{NHCOCH}_2\text{CONH}_2$.—22 g. of the crude ester were dissolved in warm 95% alcohol, filtered, and treated with an equal volume of concentrated aqueous ammonia. The amide separated on standing, after which the mixture was cooled to 0°, filtered, and washed with cold 50% alcohol. The yield of crude product was 14.6 g. Recrystallized from water, adding a few drops of ammonia to the hot, filtered solution, it forms yellow, rhombic platelets which are readily soluble in hot acetic acid, less easily in hot alcohol, water, or acetone, and difficultly in all in the cold. When rapidly heated to 215°, then slowly, it melts at 218–20° with gas evolution.

0.1262 g. subst.; 20.8 cc. N, 755 mm., 23.0°.

Calc. for $C_9H_9O_4N_2$: N, 18.83%. Found: N, 18.89%.

p-Aminomalonanilamide, $p\text{-H}_2\text{NC}_6\text{H}_4\text{NHCOCH}_2\text{CONH}_2$.—13.5 g. of the crude nitro compound were mixed to a thin paste with hot water and reduced by the ferrous sulfate and ammonia method as in previous examples. The filtrate from the reduction was acidified with acetic acid, concentrated *in vacuo* and the product filtered off. The yield was 9.7 g. Recrystallized first from water, then from 50% alcohol, using bone-black, the amide forms practically colorless, radiating masses of microplatelets which apparently contain one molecule of

water of crystallization which is not driven off at 100° *in vacuo*. When rapidly heated it sinters, then melts partly at $200-10^{\circ}$ to a purple mass which does not melt entirely below 280° . It is readily diazotized, yielding a deep red, difficultly soluble dye with R-salt, and, in aqueous suspension, gives a slowly developing wine-red color with ferrid chloride.

0.1060 g. subst.; 18.35 cc. N, 756 mm., 23.5° .

Calc. for $C_9H_{11}O_2N_3 \cdot H_2O$: N, 19.88%. Found: N, 19.83%.

p-Chloroacetylaminomalonanilamide.—5 g. of the once-recrystallized amide were chloroacetylated as in previous examples. The yield of acyl derivative was 6.5 g. Recrystallized from water, with bone-blackening, it forms sheaves of delicate hairs which are difficultly soluble in the usual solvents. Rapidly heated to 240° and then slowly, it melts with decomposition at $243-4^{\circ}$. It gives a strong Beilstein test.

0.1407 g. subst.; 18.8 cc. N, 760 mm., 23.5° .

Calc. for $C_{11}H_{12}O_2N_3Cl$: N, 15.59%. Found: N, 15.38%.

3. Derivatives of 2,4-Tolylenediamine.

2-Amino-4-acetaminotoluene Hydrochloride.—Although this substance was prepared both by Wallach and Schulze¹ and Schiff and Ostrogovich² we were unable to find a complete description of it in the literature. The salt was prepared and purified as in the case of *m*-aminoacetanilide hydrochloride (see p. 169). Starting with 324 g. of commercial 2,4-toluylenediamine, 120 g. of the hydrochloride were obtained. Recrystallized from 95% alcohol it forms delicate needles which, when rapidly heated to 260° and then slowly, decompose at $263-4^{\circ}$ with slight preliminary sintering.

0.1829 g. subst.; 0.1314 g. AgCl.

Calc. for $C_9H_{10}ON_2 \cdot HCl$: Cl, 17.68%. Found: Cl, 17.78%.

Addition of sodium carbonate to a solution of the salt gives the base, which crystallizes from water in long, brilliant needles which melt at $161-2^{\circ}$, as described in the literature.

¹ *Loc. cit.*

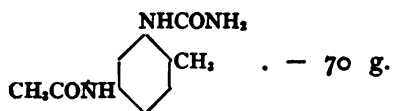
² *Ibid.*, p. 371.

2-Chloroacetyl-amino-4-acetaminotoluene.—10 g. of the above hydrochloride were dissolved in a mixture of 50 cc. acetic acid and 50 cc. saturated sodium acetate solution and chloroacetylated in the usual way. The yield of acyl derivative was 10 g. Recrystallized from 95% alcohol, it forms delicate, felted hairs which are more difficultly soluble in boiling water than in boiling alcohol and are practically insoluble in boiling benzene. Rapidly heated to 225°, then slowly, the substance softens slightly and then melts at 230–1° with slow gas evolution and reddening.

Kjeldahl: 0.1128 g. subst.; 9.4 cc. 0.1 *N* HCl.

Calc. for $C_{11}H_{13}O_3N_2Cl$: N, 11.65%. Found: N, 11.68%.

2-Methyl-5-acetaminophenylurea,



aminoacetaminotoluene hydrochloride were dissolved in 800 cc. of warm 50% acetic acid, treated with 35 g. sodium acetate, shaken until a clear solution was obtained, and then cooled to 0°. A solution of 45 g. sodium cyanate was then slowly added, with stirring and chilling. The urea separated almost immediately in practically quantitative yield. Recrystallized from water, it forms delicate, felted needles which melt with effervescence at about 240°, resolidifying and then not melting below 280°. The urea dissolves in hot water or alcohol but is almost insoluble in acetone, benzene, or chloroform.

0.1062 g. subst.; 18.5 cc. N, 762 mm., 21.0°.

Calc. for $C_{10}H_{12}O_2N_2$: N, 20.28%. Found: N, 20.29%.

2-Methyl-5-aminophenylurea Hydrochloride.—325 g. of boiling 1:1 hydrochloric acid were added to 62.5 g. 2-methyl-5-acetaminophenylurea, after which the solution was boiled 15 minutes and rapidly chilled. The precipitate was filtered off and recrystallized by dissolving in the minimum amount of hot water, cooling, and adding an equal volume of concentrated hydrochloric acid. The resulting salt was washed with 1:1 hydrochloric acid and dried. The yield was 30 g. A portion was recrystallized by dissolving in hot 85% alcohol and adding a drop of concentrated hydrochloric acid to the solution before cooling.

It forms sheaves of minute needles which soften but do not melt below 280° . The salt is soluble in water or methyl alcohol and difficultly so in hot absolute alcohol. The aqueous solution gives a slowly developing reddish color with ferric chloride.

0.1758 g. subst.; 0.1255 g. AgCl.

Calc. for $C_8H_{11}ON_2 \cdot HCl$: Cl, 17.59%. Found: Cl, 17.66%.

2-Methyl-5-aminophenylurea.—A portion of the hydrochloride was dissolved in warm water, cooled, and an excess of ammonia added. The base separated almost immediately as glistening scales. These were filtered off, washed with water, and recrystallized from water, forming star-shaped aggregates of spears. Rapidly heated to 195° , then slowly, the urea melts and effervesces at $199-200^{\circ}$, soon resolidifying and then not melting below 280° . It is difficultly soluble in cold water or 95% alcohol, hot chloroform or acetone, and readily in the first two solvents on boiling. The substance is easily diazotized, giving a dark red color with R-salt. The urea is mentioned by Strauss¹ as having been obtained by Märker, but we were unable to find a description recorded.

0.0903 g. subst.; 19.8 cc. N, 758 mm., 21.5° .

Calc. for $C_8H_{11}ON_2$: N, 25.44%. Found: N, 25.36%.

2-Methyl-5-chloroacetylaminophenylurea.—This substance was prepared as in the case of the chloroacetylaminacetaminotoluene (see p. 189). 5 g. of the aminotolylurea hydrochloride yielded 4.6 g. of the acyl derivative. Recrystallized from 95% alcohol, it forms radiating masses of delicate needles which dissolve more readily in hot acetic acid than in the other usual solvents. Rapidly heated to 220° and then slowly, it melts at $224-5^{\circ}$ with gas evolution to a liquid which gradually turns yellow. The aqueous solution, boiled with sodium hydroxide, splits off chlorine ion.

0.1080 g. subst.; 16.0 cc. N, 770 mm., 20.5° .

Calc. for $C_{10}H_{12}O_2N_2Cl$: N, 17.39%. Found: N, 17.46%.

¹ Strauss, *Ann.*, 148, 159 (1868).

THE PREPARATION OF β -CHLORO- AND β -BROMOPROPIONIC ACIDS.

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Of the chief methods to be found in the literature for the preparation of β -chloro- and β -bromopropionic acids only that involving the oxidation by nitric acid of β -chloro- and β -bromopropionaldehydes,¹ obtained by saturation of acrolein with halogen acids, seemed to offer promise for the preparation of considerable quantities of material. Although the yields claimed for this method are excellent, the necessity of working with unpleasant substances led us to seek for a more convenient procedure.

Beckurts and Otto² prepared the two acids in question by treating hydracrylic acid with hydrochloric and hydrobromic acids. Hydracrylic acid, in its turn, was obtained by Moureau³ by hydrolysis of ethylene cyanohydrin, which he prepared easily and satisfactorily by the interaction of ethylene chlorohydrin and potassium cyanide. It occurred to the writers that, starting with ethylene cyanohydrin, the two steps of saponification of the nitrile to the acid and replacement of the hydroxyl group by halogen could be accomplished simultaneously by heating the cyanohydrin with concentrated halogen acid. These expectations were confirmed. Since all of the steps involved may be rapidly and conveniently executed the authors feel that this procedure will prove to be the most satisfactory method for those who have occasion to prepare these substances.

¹ Krestovnikov, *J. Russ. Phys. Chem. Soc.*, 11, 248 (1879); Lederer, *J. prakt. Chem.*, [2] 42, 384 (1890).

² Beckurts and Otto, *Ber.*, 18, 226 (1885).

³ Moureau, *Bull. soc. chim.*, [3] 9, 426 (1893).

EXPERIMENTAL.

Ethylene Cyanohydrin, $\text{HOC}_2\text{H}_4\text{CN}$.—The only variation from Moureaux's method was that the product was purified by distillation *in vacuo*. 32 g. ethylene chlorohydrin were dissolved in 160 cc. absolute alcohol and boiled under a reflux condenser. To the boiling solution were added, drop by drop, 27.2 g. potassium cyanide dissolved in 42 cc. water. The boiling was continued for eight to ten hours. At the end the solution was filtered from potassium chloride and the filtrate concentrated to a syrup, which was fractionated *in vacuo*. The cyanohydrin boiled at 110° at 15 mm. The yield was 20 g.

β -Chloropropionic Acid.—10 g. ethylene cyanohydrin were heated in a sealed tube with 75 cc. concentrated hydrochloric acid at 100° for three hours. Ammonium chloride separated from the solution on cooling. The contents of the tube were diluted with water and extracted with ether. The ethereal extract was dried over sodium sulfate and concentrated, yielding a syrup which easily crystallized. The yield of β -chloropropionic acid so obtained was 10.5 g. Recrystallized from hot ligroin, the acid melted at $38.5\text{--}39.5^\circ$ (corr.) with slight preliminary softening. It possessed the characteristic odor and other properties of β -chloropropionic acid.

0.1964 g. subst.; 0.2560 g. AgCl .

Calc. for $\text{C}_3\text{H}_5\text{O}_2\text{Cl}$: Cl, 32.68%. Found: Cl, 32.25%.

β -Bromopropionic Acid.—10 g. ethylene cyanohydrin were boiled with 100 cc. hydrobromic acid (d. 1.49) for three hours. The mixture was cooled, diluted with water, and extracted with ether. The dried ethereal extract yielded on concentration 17 g. β -bromopropionic acid. As so obtained the substance is practically pure. Recrystallized from hot ligroin it melted at $60\text{--}61^\circ$ (corr.) and possessed the recorded properties of β -bromopropionic acid.

0.1554 g. subst.; 0.1910 g. AgBr .

Calc. for $\text{C}_3\text{H}_5\text{O}_2\text{Br}$: Br, 52.24%. Found: Br, 52.30%.

ON THE PRESENCE OF ALBUMOSES IN EXTRACTS OF THE POSTERIOR LOBE OF THE HYPOPHYSIS CEREBRI.*

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(From the Pharmacological Laboratory, Johns Hopkins University.)

(Communicated July 2, 1917.)

In recent years some very definite statements have been made in respect to the chemical nature of the active principle or principles of the hypophysis cerebri (pituitary gland). Among the claims advanced none are more sharply defined than those published by H. Fühner¹ on behalf of the chemists of the research laboratory of the Farbwerke-Hoechst Company.

From extracts of the posterior lobe of the hypophysis, which had been freed of coagulable proteids,² these investigators obtained a mixture of crystalline sulphates of high physiological activity which was quite unjustifiably named 'Hypophysin.' What misconceptions may arise in connection with the use of this term 'hypophysin'—a designation for an unknown number of substances—may be seen when we read in a treatise on organotherapy that "Hypophysin is the chemically active pure posterior pituitary hormone, marketed as a sulphate."³

Further research enabled the chemists referred to to separate their 'hypophysin' into four unnamed crystalline fractions which, in respect to physical and chemical properties, are easily distinguished, the one from the other. These fractions are described in the following words:

1. A colorless, well crystallized sulphate which easily dissolves in water with neutral reaction and is difficultly soluble in alcohol, acetone and ethyl acetate.

* Aided by a grant from The Rockefeller Institute for Medical Research.

¹ Fühner, H., *Zs. ges. exp. Medizin, Berlin*, 1, 1913, (397).

² "Vollständig von Eiweiss befreite Auszüge aus den Hinterlappen von Rinderhypophysen." Fühner, *loc. cit.*, p. 399.

³ Harrower, H. R., *Practical Hormone Therapy*, New York, 1914, p. 460. A Glossary of Terms.

It is optically active (laevo-rotatory, $[\alpha]_D = -54.02^\circ$) and carbonizes without melting when heated to a high temperature. The Pauly and biuret reactions are positive. With picric acid this substance forms a salt difficultly soluble in water.

2. There was also obtained a substance yielding a well crystallized, colorless sulphate which dissolves in water with faint acid reaction and is likewise difficultly soluble in alcohol, acetone, ethyl acetate, etc. The optical rotatory power of this preparation is $[\alpha]_D = -27.17^\circ$; it decomposes when heated to $198-200^\circ\text{C}$. and gives the Pauly and biuret reactions. Contrary to the substance described under (1) this compound forms a picrate easily soluble in water. If this substance is brought into contact with alkalies a volatile amine base is at once liberated.

3. A third substance was isolated in the form of a crystalline, faintly yellow sulphate which, to be sure, is present in only very small amount. It is easily soluble in water and methyl alcohol with faintly acid reaction, and difficultly soluble in absolute alcohol, acetone and ethyl acetate. It turns the plane of polarized light to the left; its rotatory power is $[\alpha]_D = -39.25^\circ$. On heating the substance decomposes at $185-186^\circ\text{C}$. The Pauly and biuret reactions are positive. With picric acid is obtained a salt easily soluble in water.

4. The mother liquor remaining after the fractional precipitation of the three substances just described yields, on cautious evaporation in a vacuum, a brittle, glassy, hygroscopic mass which dissolves easily in water and methyl alcohol, with difficulty in ethyl acetate and acetone. The solution of this substance shows an optical rotatory power of $[\alpha]_D = -21.26^\circ$ and gives the Pauly but not the biuret reaction. Recently it has been found possible to isolate also from the mother liquors a yellow, crystalline, neutral substance which dissolves easily in water and alcohol, with difficulty in ether, acetone and ethyl acetate. The preparation in aqueous solution rotates the plane of polarized light to the right ($[\alpha]_D = +5.99^\circ$) and gives neither the Pauly nor the biuret reaction. The decomposition temperature is 95 to 96°C . The preparation gives with picric acid a compound difficultly soluble in water.

'Hypophysin' (the mixture of substances) is stated by Fühner to represent the physiological activity of the posterior lobe in respect to blood-pressure, respiration and uterine contractility and the sum of the actions of the four crystalline fractions equals that of the undifferentiated hypophysis.

Fühner's conclusions as to the pharmacological action of the four fractions are as follows:⁴

Fraction 1 has only a slight action on the respiratory apparatus and the uterus, but shows the typical action of 'hypophysin' on blood-pressure.

⁴ Fühner, *loc. cit.*, p. 443.

Fraction 2 has a pronounced action (*ausgeprägte Wirkung*) on blood-pressure, on respiration and on the uterus.

Fraction 3 behaves qualitatively like fraction 2 but has a more marked stimulating action on the uterus.

Fraction 4 (mother liquor and crystalline part) has an action on the uterus equivalent to that of fraction 3 but only a very slight action on blood-pressure and on respiration.

From his experiments Fühner draws the conclusion that the uterus-stimulating power of the hypophysis, which is, practically speaking, the most valuable property of extracts of the gland, resides, not in one, but in various (*verschiedene*) constituents of the organ, the condition being analogous to that found to hold for ergot.

From a chemical point of view, it is especially noteworthy that fractions 1, 2 and 3 give the biuret reaction as well as Pauly's reaction and that all three are laevo-rotatory; fraction 1, physiologically the least active, has the highest rotation, $[\alpha]_D = -54.02^\circ$. It is easily demonstrated, we believe, that one or more albumoses (or polypeptids if the term is preferred) are present in the first three of these fractions and no doubt also in the fourth. We have found, as will be shown in a subsequent paper, that substances of the nature of albumoses can be isolated from many organs. It is these substances, which are themselves inactive, that give the biuret, the Pauly and other reactions of the so-called isolated principles and that account for their laevo-rotation.

The following analysis of one of the commercial preparations of the posterior lobe of the hypophysis is offered in support of our contention that all those fractions of 'hypophysin' which give the biuret reaction contain albumose. The extract called 'Pituitrin' (Parke, Davis & Co.) was used first because it could be purchased in large quantities ($\frac{1}{2}$ oz. bottles).

The contents of 10 bottles, approximately 150 cc., were extracted three times with ether to remove a preservative (chloretone) and then concentrated under an electric fan on the water bath to a volume of 10 to 15 cc. A small amount of flocculent material which separated was removed by filtration, the filtrate was diluted with an equal volume of absolute alcohol and a solution of lead acetate was added, which induced a very slight precipitation. Addition of ammonia to faint alkalinity did not materially increase the precipitate, from which it was concluded that no appreciable amount of native proteid could be present, and

that no advantage would accrue from the use of basic lead acetate, as in the procedure which will be described in a subsequent paper. An albumose, especially if of secondary nature, would escape precipitation by ammoniacal lead acetate. The alcoholic filtrate was freed from lead with sulphuric acid and the filtrate from the lead sulphate was concentrated to a small volume (a few cc.) and treated with an equal volume of saturated ammonium sulphate solution. The resulting flocculent precipitate, which was not very abundant, was washed with half-saturated ammonium sulphate solution, dried *in vacuo*, dissolved in water and again precipitated with an equal volume of saturated ammonium sulphate solution. The precipitate was again washed with half-saturated ammonium sulphate solution and treated with an excess of barium hydroxide. After filtering off the barium sulphate, the filtrate was heated on the water bath until all the ammonia had been expelled and the excess of barium was removed with sulphuric acid. The filtered solution was then concentrated to a very small volume and dropped into absolute alcohol, ether being added until no further precipitation was produced. The substance here thrown out, which was small in bulk (0.010 g.), was of less interest to us than the fraction presently to be described. It gave the Millon and biuret reactions, as well as that of Pauly. Knoop's reaction for histidine was negative.

The filtrate from the half-saturation with ammonium sulphate as described above was saturated with finely powdered ammonium sulphate, which caused the appearance of a gummy precipitate so characteristic of albumoses when treated in this manner. The precipitate was filtered off, washed with saturated ammonium sulphate solution, dissolved in water and treated with barium hydroxide in the usual manner. The solution, freed from ammonia and the excess of barium, was concentrated to a very small volume and dropped into absolute alcohol, the precipitation being completed with ether. The substance thus thrown out was collected as completely as possible and dried *in vacuo*; 0.031 g. in 1 cc. of water in a 0.5 dcm. tube showed a rotation of -1.38° , whence $[\alpha]_D = -89^\circ$. When the substance was reprecipitated with hot absolute alcohol its specific rotation was found to be $[\alpha]_D = -77.6^\circ$.

As to its properties, the substance must be classed with the secondary albumoses. It has been shown to be precipitated by saturation of its solution with ammonium sulphate. It is non-coagulable on boiling, gives the biuret reaction (hemi-biuret) very beautifully, as also Pauly's reaction, while Knoop's bromine reaction for histidine is entirely negative. This negative result with Knoop's reagent excludes the presence of histidine as an admixture in our substance.⁵ Picric acid added to an aqueous solution gives a precipitate. The ad-

⁵ Cf. Aldrich, T. B., *J. Amer. Chem. Soc.*, Easton, Pa., 38, 1915, (203).

dition of Millon's reagent gave rise to a slight turbidity, but on boiling the characteristic red color was not obtainable. In this respect there was entire agreement with a secondary albumose which we have isolated from the mucosa of the small intestine. The ninhydrin reaction was positive when made in the usual manner but we are confident that this is due to the fact that adherent amino acids were not entirely removed. To do this would have required several reprecipitations with ammonium sulphate.

The albumose here described was found to have a quite negligible action when tested with the virgin cat's uterus.

A secondary albumose which was prepared by digesting fresh thyroid glands of the pig behaved in every respect like the above substance. Its specific rotation was found to be $[\alpha]_D = -88.1^\circ$, while that of the pituitary albumose varies from -78° to -89° .

We come now to the filtrate from the complete salting out with ammonium sulphate. This was freed from ammonium sulphate in the usual manner. Reduced to a small volume, the solution was dropped into absolute alcohol, the precipitation being completed with ether as with the preceding substances. The material thus obtained was readily soluble in water and gave the usual response of pituitary extracts when tested on the isolated uterus of the virgin guinea pig. It also still gave the Pauly and the biuret reactions, though with greatly lessened intensity as compared with the original solution. It fails to give Knoop's reaction for histidine so that we must conclude that this amino acid does not exist as such in any considerable amounts, if at all, in pituitary extracts. The rotation of 0.0381 g. in 1 cc. of water in a 0.5 dcm. tube was found to be -0.52° , from which $[\alpha]_D = -27.4^\circ$. We have here a substance which may be compared with respect to physiological activity, rotation and chemical tests with Fühner's 'hypophysin' fractions 2 and 3. The solution used in the polarizing tests was saturated with powdered ammonium sulphate and again the characteristic precipitate of albumose was produced, though, naturally, it was not abundant this time. Evidently we have here the remnant of albumose which remained in solution after the first saturation with ammonium sulphate. If we were to apply this salting out to larger quantities of pituitary extract it is possible that more or less of a true peptone would be found in the filtrate. Certainly, there always remains in the filtrate, even after two saturations, a substance which gives a pink biuret reaction. Only more extended research can show to what extent this is albumose and to what extent peptone.

We believe that there is no mystery attaching to the constituents of pituitary extracts that have been shown to give positive Pauly and

biuret reactions and a negative Knoop's bromine reaction. These constituents must be classed as albumoses (and even peptones) and the German chemists are to be congratulated if they have obtained them in the form of physiologically active crystalline sulphates, as has been stated on their behalf by Fühner, even though they have failed to recognize their proteid nature.

We have made qualitative tests with other commercial pituitary extracts (Armour's Pituitary Liquid, and Solution Pituitary Extract, Mulford) and have found, as was to be expected, that albumoses can be salted out from all of them. The amount of proteid material present varies considerably in these preparations—one of them (Armour's Pituitary Liquid) appears to have only a trace of that form of proteid (coagulable proteid plus primary albumoses) which gives a precipitate with potassium ferrocyanide and acetic acid and to have practically all its biuret-yielding substance in the form of secondary albumose.

As to the total amount of biuret-yielding material present in the extract analyzed—and by this we mean the substance or substances that give the biuret reaction *immediately at room temperature* (and not, as histidine gives it, after heating)—we believe that we are close to the truth when we say that it cannot be far below 10% in weight of the total solid matter. The dry residue from five bottles of extract (73 cc.), exclusive of chloretone, was found to be 0.415 g. The amount of albumose, primary (?) and secondary, recovered from ten bottles (in the analysis described above), with a dry residue of 0.830 g., was approximately 0.050 g. The losses, at a conservative estimate, could hardly have been less than 0.025 to 0.030 g. On this basis we should have had, in the specimen analyzed close to 10% of biuret-yielding material.

It may be of interest to state here that when the dry residue of five bottles (0.415 g.) was heated in a boiling water bath for one hour in 10 cc. of 25% hydrochloric acid the biuret reaction⁶ disappeared entirely but the Pauly reaction for histidine was still obtained. The disap-

⁶ The biuret reaction was made at room temperature. Unfortunately we did not apply heat as is done when making this test for histidine. This substance was no doubt present, having been set free from the albumoses by hydrolysis, as is shown by the positive Pauly test.

pearance of the biuret reaction after boiling with hydrochloric acid can only be interpreted as due to hydrolysis of our albumose.

After having completed our examination of the American pituitary preparations we learned that the 'hypophysin' of the Hoechst chemists could be obtained in this country. We accordingly purchased two hundred 1 cc. ampullæ of this product, which is described on the labels as a "sterile solution, 1:1000, of the isolated active substances from the *glandula pituitaria*." We did not inquire if a preservative is used in its preparation, as the presence of a substance of this character would hardly interfere with the isolation of a proteose.

The residue from ten ampulles, as obtained by evaporation at a low water-bath temperature under an electric fan and subsequent drying over sulphuric acid, amounted to 0.0154 g. The dry residue from the two hundred 1 cc. ampulles would therefore have weighed 0.3080 g. One hundred and ninety cubic centimeters, that is to say, the total quantity of solution with the exception of the 10 cc. used for the estimation of the dry residue, were concentrated on the water bath under the fan to a volume of 2.5 cc.⁷ and saturated with finely powdered ammonium sulphate. The characteristic gummy precipitate of salted-out albumose immediately collected on the stirring rod and on the sides of the tube containing the solution. The precipitate was washed with saturated ammonium sulphate solution, decomposed with barium hydroxide and the freed albumose was precipitated as a "sulphate" with absolute alcohol and ether in the manner already described. Dried over sulphuric acid, the albumose thus obtained weighed 0.017 g. The reactions were those already described—a beautiful pink biuret, a positive Pauly and a negative Knoop reaction. Potassium ferrocyanide and acetic acid also failed to give a precipitate, showing that coagulable proteids and primary albumoses were not present.

The ammonium sulphate filtrate from the gummy albumose precipitate still gave a fine pink biuret reaction, as was the case also with all the American preparations under the same conditions. The addition of a drop or two of a very concentrated solution of trichloroacetic acid to this filtrate caused an immediate precipitation of gummy droplets. These give the biuret reaction with great intensity and represent a further yield of albumose with a probable admixture of peptone and traces of other substances. It may be stated in this connection that the ammonium sulphate filtrates of our American preparations also give with trichloroacetic acid a precipitate which is indistinguishable in its reaction from that obtained with hypophysin. A certain amount of albumose or peptone still remains in these ammonium sulphate filtrates even after the use of trichloroacetic acid.

The Hoechst preparation is no doubt a clean product and certainly

⁷ This also contained the redissolved residue from the 10 cc. used for the estimation of dry matter.

contains less dry residue than the products prepared in this country. We have seen that the dry residue of ten 1 c. ampulles of hypophysin was 0.0154 g. The dry residue of ten 1 cc. ampulles of Armour's Pituitary Liquid was 0.0242 g. and in its relatively smaller content in proteoses this preparation more nearly approaches hypophysin than any other examined by us. It is to be understood that we are not criticizing these products because they happen to contain more or less albumose. This in itself is of no consequence, as this albumose does not appear to be toxic.

The points that we wish to emphasize are these:

1. Carefully prepared commercial extracts of the posterior lobe of the hypophysis contain albumoses.
2. Hypophysin, stated to be a mixture of the "isolated active substances of the pituitary gland," is likewise contaminated with albumoses.
3. All claims in respect to the isolation of pure principles, as made by the Hoechst chemists, must be looked upon, in view of our findings, as being without foundation.

Other considerations also lend support to the last statement.

One who is familiar with the high activity for the virgin uterus of fresh extracts of the hypophysis can only agree with Fenger when he asserts that the as yet unknown constituent of this gland which affects the uterus so powerfully cannot be less potent than β -imidoazoly-lethylamine, and may be even more powerful. Fenger says that an acidulated methyl alcohol extract of the posterior lobe of the hypophysis, for which no claim to chemical purity can be advanced, "showed a uterine-contracting power somewhat stronger than pure β -I."⁸ If we examine the tracings given by Fühner in his experiments with the Hoechst products, experiments in which quantities varying from 0.05 to 0.5 mgm. were tested on the guinea pig's uterus in a 100 cc. bath of Locke's solution, it will be seen that these products are much weaker than β -imidoazoly-lethylamine. Here again is evidence that the crystalline salts of the Hoechst chemists represent mixtures of active and inactive principles and not pure chemical individuals.

Further evidence that Hypophysin does not consist of chemically pure principles is given by the pharmacological tests made with it in

⁸ Fenger, F., *J. Biol. Chem., Baltimore*, 25, 1916, (417).

this laboratory. Dr. D. I. Macht has kindly compared the oxytocic strength of the preparation with that of Armour's Pituitary Liquid, this having been selected from among the American products because it most nearly approaches Hypophysin in respect to dry matter and a low albumose content. He reports that the Armour product, which makes no pretense of being a pure chemical principle, is "several times more powerful in its action on the virgin uterus of the guinea pig than Hypophysin." There is no reason to assume that the Hypophysin used in Dr. Macht's tests had lost any of its original strength as the labels on the packages give no hint of instability or loss of strength with time.

The question naturally arises whether the albumose or other proteose here shown to be present in all active pituitary extracts is not itself the uterine stimulant. The secondary albumose which was isolated by us from "Pituitrin" was practically devoid of an oxytocic action, as has already been stated. Investigations on the bio-chemistry of the intestinal and gastric mucosa which we hope soon to publish also lend no support to the theory that pituitary extracts contain an active albumose. We have prepared a water-soluble powder from this mucosa which is highly active for the guinea pig's uterus (1:1,000,000) and for the intestinal strip (1:250,000), which induces a distinct rise of blood-pressure in the cat, and which in respect to its chemical reactions, its behavior towards ammonium sulphate and polarized light, *is indistinguishable from a diluted pituitary extract*. The similar behavior in these several respects of gastric and intestinal 'motiline' solutions and pituitary extracts first led us to suspect that these latter also contain albumoses. Now, in the case of these intestinal preparations, we have had sufficient material on hand for the application of purification processes. *We finally emerged with a secondary albumose which was entirely devoid of oxytocic, pressor, depressor or secretory action*. It is this experience, together with our discovery that an inactive albumose can be prepared from the ordinary pituitary extracts, as already stated, which fortifies us in our belief that the proteoses of the gland have nothing whatever to do with the physiological activity of the organ.

It is not our purpose to consider here the literature⁹ pertaining to

⁹ Cf. Ott, Enriquez and Hallion, Zuelzer, Weiland, Köhler and others.

the 'peristaltic hormones' that are known to occur in almost all, if not all, organs of the body. It is worthy of note, however, that an extract of the gastric or intestinal mucosa can be prepared, as we have already stated, which has a pressor action for the circulation and a marked oxytocic power in a concentration of 1:1,000,000. This powerful action points strongly to the conclusion that here also, as in the case of pituitary extracts, we are dealing with a motiline which, in a state of chemical purity, would be fully as active as β -imidoazolyethylamine. And this again leads us to the supposition that the oxytocic principle (or motiline) of the hypophysis is not a hormone or substance specific to this organ, but is rather a widely distributed substance, everywhere the same, which may have its origin in the various tissues, in the gastric or intestinal mucosa, or which may be absorbed as such from among the products of digestion. We hope that our discovery of the contaminating and difficulty separable proteoses in physiologically active extracts will pave the way to the solution of these problems.

We cannot conclude this communication without adding a few words in regard to the presence of proteoses in the various tissues of the body. Proof of their existence in pituitary extracts has been given and reference has been made to their presence in gastric and intestinal extracts. By the use of certain methods which will shortly be described in detail we have found that a secondary albumose (to name only a single proteose which is sharply differentiated from all native proteids and primary proteoses) can be isolated in small amounts from all of the cellular tissues of the body thus far examined. Skeletal muscle appears to contain albumose in the smallest amount, gastric and intestinal mucosa contain it even after four days' starvation, *much more during digestion of a meat meal*, while organs like the thyroid gland contain much more, weight for weight, than skeletal muscle. We have not as yet been able to isolate definitely a true proteose of any sort from the plasma of the blood, though able to show that the cellular elements of the blood yield a readily demonstrable amount of albumose.

It was not originally our purpose to study these proteoses or to isolate them from the various tissues, but finding them always present in our final products whenever we attempted to isolate certain 'hor-

mones,' such as the intestinal motiline and secretine, even when our methods of treating the tissues could not have produced them, we were forced to undertake a study of methods for their separation from the hormones. A future communication in these PROCEEDINGS will deal with this question.

CONCLUSIONS.

1. Secondary albumoses and possibly peptones (or polypeptids if the term is preferred) were found to be present in all of the therapeutically used extracts of the posterior lobe of the hypophysis cerebri that were examined. To what extent the proteose content of the gland may have been increased by autolysis or by processes incidental to the manufacture of the extracts it is impossible for us to state. We believe, nevertheless, that the perfectly fresh, bloodless glands yield proteoses, inasmuch as we have actually isolated such substances from the thyroid gland and other organs when taken from the animal immediately after bleeding it to death.

2. The 'Hypophysin' of the Farbwerke-Hoechst Company is not, as claimed for it, "a solution of the isolated active substances of the pituitary gland" but a mixture of albumoses (and possibly peptones) with varying and unknown amounts of active and inactive constituents of the gland. The albumoses present in 'Hypophysin' account fully for the chemical reactions (such as the biuret and the Pauly reactions and the left-handed rotation) which are stated to characterize the pretended active principles. The albumoses as separated from pituitary extracts are devoid of action upon the uterus. In view of the facts here presented it must be evident that the active principles of the hypophysis cerebri have not yet been isolated as chemical individuals.

CICATRIZATION OF WOUNDS.

V. NEW MATHEMATICAL EXPRESSION OF CICATRIZATION.

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A new formula with two equations gives in mathematical terms Carrel and Hartmann's law:¹ "the rate of cicatrization diminishes at the same time as the size but less rapidly."

In the time dt , the area cicatrized, dS , is proportional to S :

$$dS = -\lambda S dt \quad \text{or} \quad \frac{dS}{dt} = -\lambda S$$

λ is positive and the formula shows that the rate, $\frac{dS}{dt}$, decreases with S .

By integration we get

$$\int \frac{dS}{S} = -\lambda \int dt$$

$$(1) \quad \text{Log}_e S = -\lambda t + \text{Log}_e S_0 \quad \text{or} \quad S = S_0 e^{-\lambda t}$$

where S_0 is the initial area.

If the coefficient λ is constant, the law of cicatrization can be expressed by simple logarithmic formula.

The rate of cicatrization decreases less rapidly than the size; that is, λ is not constant and must increase slightly when the area decreases. In the time dt the variation of λ , $d\lambda$, is proportional to λ :

$$d\lambda = \mu \lambda dt \quad \text{or} \quad \frac{d\lambda}{dt} = \mu \lambda$$

If μ is positive, the equation indicates that λ increases because the derivative $\frac{d\lambda}{dt}$ is positive.

¹ Carrel, A., and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

By integration we get

$$\int \frac{d\lambda}{\lambda} = \mu \int dt$$

$$(2) \quad \text{Log}_e \lambda = \mu t + \text{Log}_e \lambda_0 \quad \text{or} \quad \lambda = \lambda_0 e^{\mu t}$$

where λ_0 is the initial value of λ . λ is calculated by equation (2) and with this value of λ we can obtain S by the equation (1).

The two coefficients λ_0 and μ may be determined to make the values calculated and observed correspond.

The area at any time can be obtained immediately without calculating the intermediate areas.

CICATRIZATION OF WOUNDS.

VI. BACTERIOLOGICAL ASEPSIS OF A WOUND.

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(Received for publication, February 9, 1917.)

In a previous communication¹ it has been shown that even a slight infection prevents normal cicatrization. Experiments on cicatrization must be carried out on surgically aseptic wounds. The degree of asepsis can easily be obtained by the method already described.² Surgical asepsis differs widely from bacteriological asepsis. Disinfected wounds which unite by first intention still contain numerous bacteria, particularly *Micrococcus epidermidis albus*. However, the experiments carried out by Carrel and Hartmann have shown that the new method of wound sterilization frequently leads to bacteriological asepsis.

The object of the following experiments was to determine to what degree wounds irrigated with Dakin's solution or treated with chloramine paste become bacteriologically aseptic.

Preventive Action of Dakin's Hypochlorite Solution and of Chloramine-T.

In the first series of experiments we undertook to ascertain what quantity of Dakin's solution or of chloramine-T is necessary to retard or prevent the growth of staphylococcus. This preliminary experiment was necessary in order to determine whether the quantity of antiseptic taken from the surface of the wound at the same time as the secretions was strong enough to inhibit the growth of the bacteria contained in the secretions.

¹ Carrel, A., and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

² Carrel, A., and Dehelly, G., *Le traitement des plaies infectées*, Paris, 1916.

To 5 cc. of broth are added 0.1 to 2 cc. of Dakin's solution or of chloramine-T solution. A drop of a 24 hour broth culture of staphylococcus is then added. The staphylococcus used was obtained from a case of suppurative arthritis of the wrist treated during the preceding 15 days with Dakin's solution. Tables I to IV give the results of the experiments. They show that the power of Dakin's solution to restrain growth becomes evident after 12 hours if more than 0.4 cc. is used. In the same way, 0.3 cc. of 1 per cent chloramine-T also retards the growth. This power is relatively weak. The amount of Dakin's solution or of chloramine-T necessary to prevent completely the growth of the staphylococcus in 5 cc. of broth is, therefore, relatively large. 2 cc. of Dakin's solution or more than 1 cc. of 1 per cent chloramine-T are necessary for sterilization of the media.

TABLE I.

Antiseptic Properties of Dakin's Solution. First Test.

In 5 cc. of broth.	Cc. of Dakin's solution.																Control.
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	2.0	
<i>days</i>																	
1	+	+	+	+	+	+	Weak.	?	-	-	-	-	-	-	-	-	+
2	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+

TABLE II.

Second Test.

In 5 cc. of broth.	Cc. of Dakin's solution.								Control.
	0.9	1.0	1.1	1.2	1.3	1.4	1.5	2.0	
<i>hrs.</i>									
6		-	-	-	-	-	-	-	Weak.
12		-	-	Weak.	-	-	-	-	+
24	+	-	-	+	Weak.	-	-	-	+
48	+	-	+	+	+	+	+	-	+

TABLE III.
Restraining Power.

In 5 cc. of broth.	Cc. of Dakin's solution.										Control.
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	
<i>hrs.</i>											
6	+	Weak.	Very weak.	?	—	—	—	—	—	—	+
12	+	+	Weak.	+	Weak.	—	—	—	—	—	+
24	+	+	+	+	+	+	+	—	+	—	+
36	+	+	+	+	+	+	+	+	+	+	+

TABLE IV.
Antiseptic Properties of 1 Per Cent Chloramine-T.

In 5 cc. of broth.	Cc. of chloramine-T.																Control.
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	2.0	
hr.																	
6	Weak.	Weak.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Weak.
12	+	+	?	—	—	?	—	—	—	—	—	—	—	—	—	—	+
18	+	+	Weak.	—	?	Weak.	—	—	—	?	Weak.	—	—	—	—	—	+
24	+	+	+	?	+	+	?	+	—	Weak.	—	—	—	—	—	—	+
36	+	+	+	+	+	+	+	+	—	+	—	—	—	—	—	—	+
48	+	+	+	+	+	+	+	+	—	+	—	—	—	—	—	—	+
3 days	+	+	+	+	+	+	+	+	—	+	—	—	—	—	—	—	+

These experiments show that the small quantity of antiseptic removed with the bacteria by the platinum loop does not affect the growth of the cultures described below.

Bacteriological Examination of Surgically Aseptic Wounds.

As soon as films taken on several consecutive days showed that no bacteria were present, cultures were made from different parts of the wound. Before taking the cultures the irrigation was suspended for 2 hours, and in cases in which the chloramine paste had been used, all trace of the paste was carefully removed with distilled water and the wound was swabbed with sterilized gauze. It is advisable when taking the films to scrape the surface of the wound lightly with a platinum loop, care being taken to avoid making the granulations bleed.

TABLE V.

Results of Experiments.

Case No.	Wound.	Treatment.	Duration of treat-	Result of culture.	Bacteria recovered.	Result.
			ment.			
			days			
1	Complicated fracture of right humerus.	Dakin's solution.	7	+	<i>B. pyocyaneus</i> and various cocci.	Closure and suture 6 days after culture. Healing <i>per primam</i> .
2	Flesh wound of right knee.	"	7	+	<i>Staphylococcus albus</i> and an unknown bacillus.	"
3	Flesh wound of left leg.	"	7	+	<i>Micrococcus epidermidis albus</i> and <i>B. pyocyaneus</i> .	"
4	Flesh wound of right thigh.	"	8	+	<i>Enterococcus</i> , <i>Staphylococcus albus</i> , and various diplococci.	Closure and suture 4 days after culture. Healing <i>per primam</i> .
5	Perforated wound with fracture of right humerus.	"				
	(a) External wound.		8	—		
	Anaerobic culture.		11	—		
	Subculture.		11	—		
	(b) Internal wound.		8	+	<i>Micrococcus epidermidis albus</i> .	Closure and suture on day of subculture. Healing <i>per primam</i> .
6	Complicated fracture of neck of left femur.	"	12	+	<i>Staphylococcus aureus</i> , <i>Micrococcus epidermidis albus</i> , and various diplococci.	Closure and suture 13 days after culture. Healing <i>per primam</i> .
7	Complicated fracture of right tibia.	"	42	+	<i>Staphylococcus aureus</i> and <i>albus</i> .	Closure and suture on day of culture. Sloughing on 2 cm. of the suture. Surface granulation.
8	Surface wound following an old fracture of tibia.	Dakin's solution	109	—		
	Anaerobic culture.	and	112	—		
	Subculture.	chlor-	112	—		
	Second culture.	amine-T.	117	+	<i>Micrococcus albus</i> and bacillus like <i>Proteus vulgaris</i> .	

TABLE V—*Continued.*

Case No.	Wound.	Treatment.	Duration of treatment.	Result of culture.	Bacteria recovered.	Result.
9	Wound of dorsal surface of foot following an old fracture of fifth metatarsal. (Wound heavily infected; gaseous gangrene.)	Dakin's solution and chloramine-T.	109 days	+	<i>Staphylococcus aureus</i> , <i>B. pyocyaneus</i> , and an unknown bacillus.	Surface granulation.
10	Flesh wound of calf.	"	117	+	<i>Staphylococcus aureus</i> and <i>albus</i> and an unknown bacillus.	"
11	Wound of dorsal surface of foot above fracture of metatarsus. Anaerobic culture. Subculture.	"	37	—		"
12	Complicated fracture of fourth metatarsal. Anaerobic culture. Subculture. Second culture.	"	40 40 23	— — —		"
13	Open wound of leg after ligation of popliteal and suture of posterior tibial arteries.	Dakin's solution.	20	+	<i>Micrococcus epidermidis albus</i> .	Closure and suture on day of culture. Healing <i>per primam</i> .
14	Flesh wound of right arm. Anaerobic culture. Subculture.	"	8 11 11	— — —		Surface granulation.
15	Large wound with fracture of shoulder blade. (a) Upper part of wound. (b) Lower part of wound. Subculture.	"	16 16 19	+ — —	<i>Staphylococcus albus</i> .	After slight reaction, inflammation on upper border of wound, redness for 3 days, and small hematoma surrounding the suture.

TABLE V—*Concluded.*

Case No.	Wound.	Treatment.	Duration of treatment.	Result of culture.	Bacteria recovered.	Result.
			days			
16	Flesh wound of calf with lymphangitis.	Dakin's solution.	19	+	<i>Micrococcus albus</i> and an unknown diplococcus.	Closure and suture on day of culture. Healing <i>per primam</i> .
17	Wound of left arm with comminuted fracture of humerus.	Dakin's solution and chloramine-T.	19	+	<i>Streptococcus</i> and <i>Staphylococcus albus</i> .	Surface granulation.
18	Flesh wound of right leg.	Chloramine-T.	20	—		"
	Anaerobic culture. Subculture.		23	—		
19	Wound of left hand with complete destruction of fourth and fifth metacarpals.	"	23	—		
			23	+	<i>Staphylococcus aureus</i> and <i>albus</i> .	"
20	Wound of left knee following arthroto-my.	Dakin's solution and chloramine-T.	98	—		"
	Anaerobic culture. Subculture. Second culture.		101	—		
			101	—		
			110	—		

The tubes of broth were inoculated and incubated for 15 days at 36°C. If the culture remained sterile, a subculture was made on the 3rd day. At the same time, a fresh film was taken at the surface of the wound for an anaerobic culture in glucose broth with an upper layer of paraffin oil. Broth has proved an excellent medium for the bacteria found in wounds. Twenty cases were subjected to the usual treatment with Dakin's solution or chloramine paste (Table V).

SUMMARY.

Of twenty infected cases treated with Dakin's solution or chloramine paste, seven were bacteriologically sterile. This proves that, in general, 35 per cent of the cases thus treated become bacteriologically aseptic. This degree of asepsis is not necessary in order to suture the wound, the absence of bacteria in films being sufficient. Complete sterilization of wounds, can, therefore, now be accomplished.

CICATRIZATION OF WOUNDS.

VII. THE USE OF CHLORAMINE-T PASTE FOR THE STERILIZATION OF WOUNDS.

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(Received for publication, February 9, 1917.)

It has been shown in a previous communication¹ that a wound cicatrizes rapidly if the surface is sterile. If it is more or less infected, the rate of cicatrization is slow or the wound enlarges. In order to obtain a convenient method for the sterilization of wounds we have endeavored to prepare an antiseptic paste which will retain its aseptic properties.

It has been found¹ that ointments and other fatty substances are inefficient when applied to wounds, because the bacteria and antiseptic are covered with fatty material which isolates them from each other and permits the bacteria to multiply freely. Hence the antiseptic paste must be soluble, and the bactericidal agent must be embodied in a substrate suitably chosen so that the whole constitutes a system physically homogeneous. On the other hand, to enable the antiseptic to act continuously the base should be absorbed slowly by the tissues in order to renew the surface of contact constantly. Neutral sodium stearate was used for this purpose because of the facility with which it is made antiseptic and also because it is not injurious to the tissues. It is well known that the slightly soluble sodium soaps, far from being irritating agents, are, on the contrary, soothing. Moreover, they give pastes sufficiently plastic for the dressing of wounds. One of Dakin's chloramines was selected as the bactericidal agent. After many trials we have used the following formula.

¹ Carrell, A., and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

Neutral sodium stearate.....	86 gm.
Chloramine-T.....	4-10 "
Distilled water.....	1,000 cc.

Of the less soluble sodium soaps it is essential to choose those derived from saturated fatty acids and not having double ethylene linkages. The presence of such groups which readily take up the elements of hypochlorous acid (HClO) causes a rapid disappearance of chloramine. On the other hand, stearic acid is a product of sufficient purity and is easily procured; its sodium salt obtained by boiling the calculated amount diluted with caustic soda is aseptic.

We have tried some pastes less concentrated in stearate, but they separate into two portions; the lower part is watery, and the upper portion richer in stearate so that it has the concentration indicated above, which is a minimum.

We have chosen as an antiseptic to combine with the sodium stearate one of the substances studied by Dakin, known as chloramine-T, which is the sodium salt of toluene sodium *p*-sulfochloramide. The reasons for choosing this substance were its high bactericidal power, the absence of caustic action on the skin, the possibility of an exact estimation of its strength, and its stability at a high temperature, which allows the substances to dissolve in a boiling solution of stearate. The question of using sodium hypochlorite was not considered because this product changes rapidly under the influence of heat, and especially because of the sensitiveness of soap solutions to the action of electrolytes.

Several trials were made with various proportions of chloramine-T. 20 gm. per kilo seem to make the paste irritating, thus rendering it useless. 15 gm. per kilo are tolerated by the wounds but are slightly irritating to the skin and congest the granulations, giving them a purple color. 10 gm. per kilo cause neither irritation nor pain; the wounds tolerate application for weeks. At this concentration the bactericidal action is strong enough to disinfect surface wounds completely, as rapidly as Dakin's solution (sodium hypochlorite 0.50 per cent). At the level of contact of the paste and tissues a thick greenish liquid gradually forms, which is apparently the result of the action of the secretion of the wound upon the paste. Bacteriological examination shows the secretion to be sterile, but it is

important to wash it away every day with neutral sodium oleate before making a fresh application. For wounds in the process of cicatrization, paste with 10 gm. of chloramine-T per kilo seems to retard the repair slightly. If the percentage of chloramine-T is further decreased, the antiseptic action decreases proportionately. With 4 gm. per kilo the action on infected wounds is extremely weak, but those that have been disinfected remain sterile and their cicatrization is normal.

The preparation of chloramine paste is as follows: Boil a liter of distilled water and add 80 gm. of stearic acid. When this has melted, gradually add enough caustic soda to saponify the fatty acid and after complete solution add 4 to 10 gm. of chloramine-T, according to the concentration desired. The mixture is then placed in a mixing machine and shaken until thoroughly cooled. The paste is a smooth snow-white cream. Microscopic examination shows that it is composed of a compact felting of fine needles retaining in capillary suspension a colorless liquid. The sodium stearate is slightly soluble at a cold temperature and produces this crystalline felting which retains in its interstices the antiseptic solution. The paste can be kept either in low glass jars or tin tubes, as tin is not corroded by chloramine-T.

The principal disadvantage of this paste is its poor power of preservation; numerous trials showed that 10 per cent of chloramine-T disappeared per month. Substances which might have rendered the paste more stable were either inefficient or lessened its keeping properties. The stability of the paste is limited by the stability of the solution of chloramine-T because the antiseptic is in solution in the paste.

CONCLUSION.

Dakin's toluene sodium *p*-sulfochloramide, mixed with sodium stearate, forms a paste sufficiently active and stable to be used in the treatment of wounds.

CICATRIZATION OF WOUNDS.

VIII. STERILIZATION OF WOUNDS WITH CHLORAMINE-T.

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(Received for publication, February 23, 1917.)

In a previous article¹ it was shown that the presence of bacteria at the surface of a wound retards the normal process of cicatrization. According to the nature and size of the infection, the curve representing cicatrization deviated from the calculated curve.² In order to investigate the substances which are capable of influencing tissue repair, it is, therefore, imperative that the wound should be kept in an aseptic condition. No specific influence on the progress of healing could be attributed to the substance experimented with unless the possible action of infection was entirely eliminated.

Sterilization of a wound is easily effected by the application of Dakin's hypochlorite solution at the surface of the tissues under appropriate conditions of concentration and duration.³ In the experiments to be described, it was attempted to simplify the method by substituting for the instillations of Dakin's hypochlorite solution a paste designed gradually to yield up to the tissues one of Dakin's chloramines contained therein. Investigations were undertaken to ascertain whether this paste would be able to keep a sterile wound in an aseptic condition, as well as to sterilize an infected wound, and whether it would retard tissue repair.

Chloramine Paste.

Some unpublished experiments of Dakin had shown the necessity of avoiding fatty substances in the composition of paste intended for the sterilization of wounds.

¹ Carrel, A., and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

² du Notty, P. L., *J. Exp. Med.*, 1916, xxiv, 451.

³ Carrel, A., and Dehelly, G., *Le traitement des plaies infectées*, Paris, 1916.

Dakin mixed with vaseline or lanoline certain antiseptic substances which are insoluble in water. Once these substances were applied to the wounds, their bactericidal power became almost negligible. Carrel also experimented with aqueous solutions of antiseptic substances mixed with lanoline, but they failed to have an influence on the infected wounds. It appeared that the active substance remained in the lanoline and exerted no effect upon the bacteria. Carrel next combined certain antiseptic substances in agar, but found that the consistency of the agar rendered it unsuitable for practical use. The cakes yielded to the tissues the liquid they contained, but they were too brittle and easily destroyed.

It was concluded from these experiments that, in order to be efficient, the bactericidal substance would have to be combined with a fat-free substance capable of being moulded exactly to the surface of the wound. At this point Daufresne⁴ succeeded in preparing a paste composed of sodium stearate and of toluene sodium *p*-sulfochloramide, the bactericidal properties of which had been discovered by Dakin and which is named chloramine-T for convenience. This paste is sufficiently firm not to flow away when applied to a wound, and yet fluid enough to be moulded to the anfractuosités of a granulating surface or of a fractured bone. The paste contains 8 per cent of sodium stearate and 4 to 15 parts per 1,000 of chloramine-T.

The paste is placed in sterile glass receptacles from which it is withdrawn at the time of dressing by means of wooden spatulas which have been sterilized in the autoclave. It is also preserved in tin tubes, to the ends of which is attached a rubber tube ending in a tapering glass tube. By means of the tube the paste can be injected into deep wounds or fistulas.

Technique for the Application of the Chloramine Paste.

The chloramine paste is designed to maintain in an aseptic condition wounds which have already been disinfected, or to sterilize slightly infected wounds. It should only be applied to wounds which yield small quantities of secretion, have little or no necrotic tissue, and little or no infection.

Neutral sodium oleate is poured on to the wound and the surrounding skin from a flask with a small opening. The granulations, the epithelial edges, and the skin are gently swabbed with a piece of absorbent cotton attached to a forceps. By this means an excellent

⁴Daufresne, M., *J. Exp. Med.*, 1917, **xxvi**, 91.

cleansing process is effected. The patient should feel no pain; any suffering indicates either that the sodium oleate is incorrectly prepared or that the cleansing is imperfectly carried out. The sodium oleate is next removed with a plug of cotton soaked in water, and the surface of the skin is dried by carefully applying a compress of absorbent gauze.

A sufficient quantity of chloramine paste is withdrawn from the receptacle by means of a sterilized wooden spatula and applied to the surface of the wound to the thickness of at least 1 cm. It should cover not only the granulations, but also the epithelial edges and part of the surrounding skin. If the wound is deep and anfractuous the tube containing the chloramine paste is introduced into the opening, and sufficient chloramine paste is expressed to fill the cavity. But no pressure should be applied during the process.

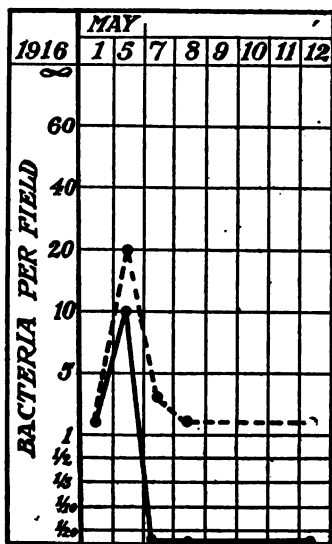
A compress of dry gauze, which should be much larger than the wound itself, is next placed over the chloramine paste. The compress is applied to the surface of the skin and attached to it by means of two or three strips of adhesive plaster. It is important that the gauze should be placed exactly over the wound, for if the bandage is shifted the gauze will introduce bacteria from the surrounding skin on to the surface of the granulations and reinfection will ensue. Above the gauze is placed a piece of absorbent cotton enveloped in gauze. The dressing must not be compressed by bandages and should be renewed every 24 hours. The wound is washed out with sodium oleate every day or two, depending on the condition of the skin. The application of chloramine should be painless; any sensation of pain signifies a technical error on the part of the surgeon.

The bacteriological condition of the wound is examined every day in film preparations of secretions taken from various parts of the wound.

Effect of a Paste Containing 4 Parts per 1,000 of Chloramine-T upon the Bacteriological Condition of an Aseptic or Slightly Infected Wound.

The influence of sodium stearate containing 4 parts per 1,000 of chloramine-T was first tested on surface wounds which had been rendered almost aseptic by instillations of Dakin's hypochlorite solution.

In the first experiment a comparison was made of the effect on the bacteriological condition of a slightly infected wound of sodium stearate alone, and of sodium stearate containing 4 parts per 1,000 of chloramine-T.

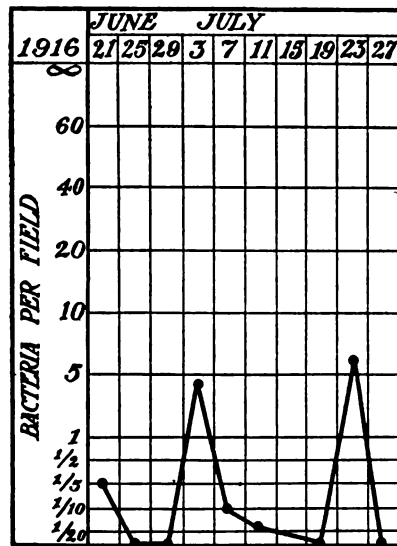


May 8. The upper part of the wound is aseptic; the lower half contains 1 bacterium per field. Same dressing.

May 12. The upper half has no bacteria, and the lower half 1 per field.

This observation shows, on the one hand, that sodium stearate has no effect on a slightly infected wound, and, on the other hand, that sodium stearate containing 4 parts per 1,000 of chloramine-T produces surgical asepsis. The bacteria disappeared completely from the films taken from the portions of the wound treated with chloramine-T, whereas they were present in all the films from the part not so treated.

In the following experiments it was attempted to maintain in an aseptic condition wounds which had been rendered surgically sterile at the beginning of treatment.



TEXT-FIG. 2. Experiment 2. Case 488. Preservation of asepsis in a wound by treatment with chloramine-T paste, 4 parts per 1,000. Wound at back of right leg. Two slight reinfections.

Experiment 2. Case 488. Slight Reinfection of a Wound Treated with Chloramine-T, 4 Parts per 1,000.—Wound at back of right leg. Treated with sodium hypochlorite solution.

June 17, 1916. The wound contains but 1 bacterium in 5 fields. Dressing with chloramine-T, 4 parts per 1,000.

June 21. 1 bacterium in 5 fields (Text-fig. 2).

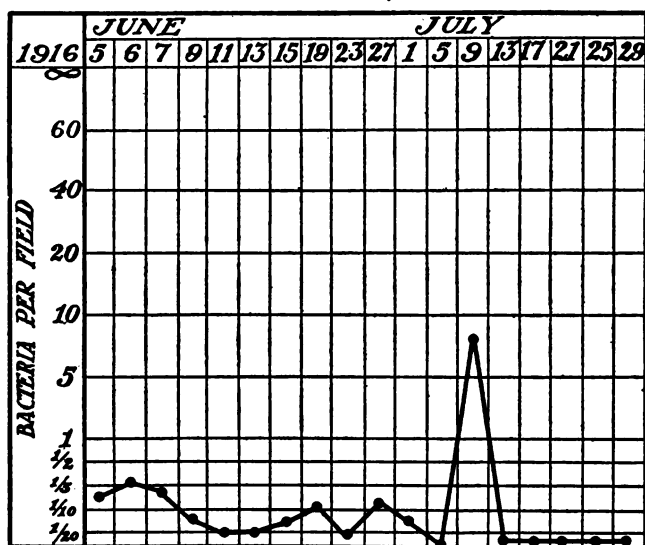
June 25-29. No bacteria.

July 3. Reinfection; 4 bacteria per field.

July 7-19. 1 bacterium in 20 fields; sometimes none.

July 23. Slight reinfection; 6 bacteria per field.

July 27. No bacteria; complete healing.



TEXT-FIG. 3. Experiment 3. Case 491. Effect of chloramine-T, 4 parts per 1,000, upon the asepsis of a wound.

Experiment 3. Case 491. Wound Kept Aseptic with Chloramine-T, 4 Parts per 1,000. Slight Reinfection.—Wound on inner side of right thigh.

Apr. 13, 1916. Wound is covered with necrotic tissue. Moderate infection. Sterilization with sodium hypochlorite.

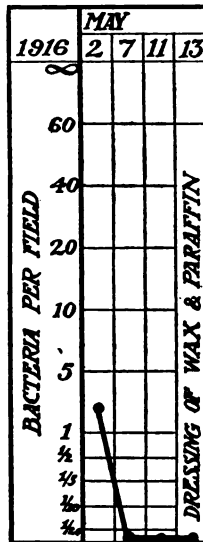
June 3. The wound is surgically sterile.

June 4. Wound is granulating and still shows small sections of necrotic aponeurosis. In these parts alone about 3 bacteria per field are found. The major portion of the wound is covered with surgically sterile granulations. Washing with neutral sodium oleate; dressing with 4 parts per 1,000 of chloramine-T.

June 5-15. The wound is dressed in the same manner and remains surgically sterile (Text-fig. 3).

July 9. Reinfection of wound; 8 to 10 bacteria per field. Dressing with chloramine-T, 10 parts per 1,000.

- July 13. Wound is sterile.
 July 14. Chloramine dressing, 4 parts per 1,000.
 July 13-29. Wound has remained sterile.



TEXT-FIG. 4. Experiment 4. Case 450. Effect of chloramine-T, 4 parts per 1,000, upon the asepsis of a wound.

Experiment 4. Case 450. Sterilization of a Slightly Infected Wound and Preservation of Asepsis with Chloramine-T, 4 Parts per 1,000.—Wound on outer surface of right leg; sterilized with sodium hypochlorite.

May 2, 1916. The secretions contain 2 bacteria per field (Text-fig. 4).

May 3. Wound is washed with neutral sodium oleate; dressing with chloramine-T, 4 parts per 1,000.

May 7. All the bacteria have disappeared.

May 13. Grafts of skin are applied to the granulations and the wound is dressed with wax and paraffin.

Experiment 5. Case 327. Reinfection of a Sterile Wound Treated with Chloramine-T, 4 Parts per 1,000.—Wound in the antero-external region of the left thigh; sterilized with sodium hypochlorite and chloramine-T solution.

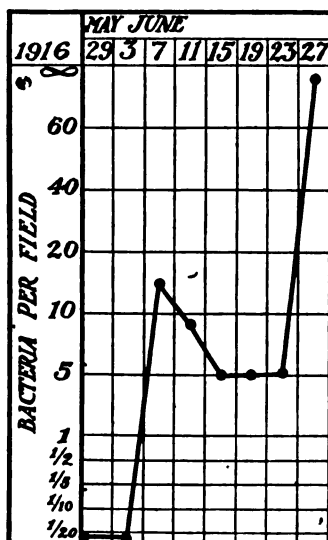
May 25, 1916. The wound, which is 25 sq. cm. in area, is surgically sterile. Dressing with chloramine-T, 4 parts per 1,000.

May 29. Wound sterile (Text-fig. 5); same dressing.

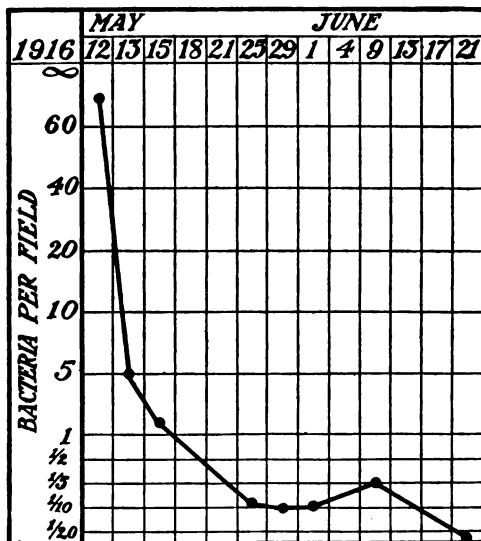
June 3. Wound sterile; same dressing.

June 7. Reinfection; same dressing.

June 27. Large number of bacteria.



TEXT-FIG. 5. Experiment 5. Case 327. Reinfection of an aseptic wound in spite of treatment with chloramine-T, 4 parts per 1,000.



TEXT-FIG. 6. Experiment 6. Case 445. Infection of cutaneous origin of a smooth and granulous wound in the foot. Sterilization with chloramine-T, 4 parts per 1,000.

Experiment 6. Case 445. Sterilization with Chloramine-T, 4 Parts per 1,000, of a Small, Slightly Infected Surface Wound.—Wound in the dorsal region of the foot.

May 12, 1916. Wound shows grayish granulations and the secretions contain approximately 100 bacteria per field (Text-fig. 6). Dressing of chloramine-T, 4 parts per 1,000.

May 29. Wound is surgically sterile and remains sterile through June 21, at which date complete healing is effected.

It has thus been shown that wounds in the fleshy regions could be maintained in a condition of surgical asepsis with sodium stearate containing 4 parts per 1,000 of chloramine-T. It was also possible to sterilize wounds presenting a slight infection of cutaneous origin. But in other cases the 4 parts per 1,000 of chloramine-T failed to maintain the asepsis. In Experiment 5 cutaneous reinfection developed in spite of the daily application of chloramine-T. The same occurred in Experiments 2 and 3, in which bacteria reappeared. It is probable, therefore, that the concentration of chloramine-T was too weak.

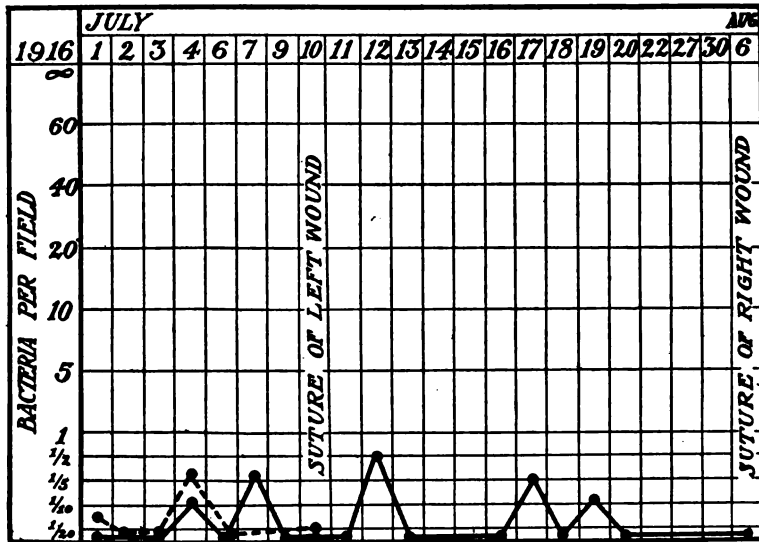
Effect of a Paste Containing 10 Parts per 1,000 of Chloramine-T upon the Bacteriological Condition of an Aseptic or Slightly Infected Wound.

Wounds, in some cases accompanied by fracture, were sterilized with Dakin's solution, and then dressed with chloramine-T, 10 parts per 1,000.

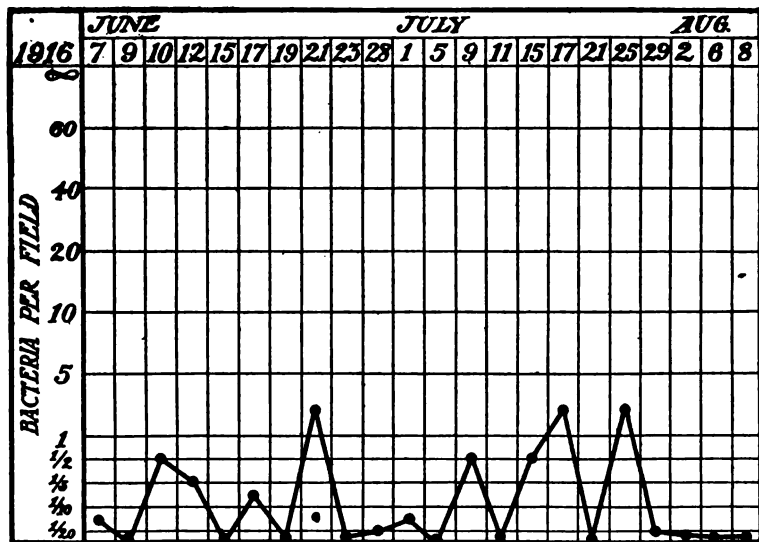
Experiment 7. Case 548. Preservation of Asepsis with Chloramine-T, 10 Parts per 1,000, of Two Penetrating Wounds in the Region of the Buttocks, Accompanied by Extensive Tissue Detachment.—Deep wounds in both buttocks caused by a projectile which entered the right thigh, came out between the buttocks in the anal region, and penetrated the left buttock, where it produced extensive muscular detachment. The large wounds and the intramuscular channels were sterilized with Dakin's hypochlorite solution.

July 1, 1916. The right wound has a surface area of 4 sq. cm., and the left of 10 sq. cm. These wounds communicate by means of deep anfractuous passages with the wounds located in the vicinity of the anus. Chloramine-T, 10 parts per 1,000, is carefully injected into the channels. This dressing is repeated daily.

July 6. The lower portion of the left wound has closed (Text-fig. 7). The deep channels healed spontaneously; the cavity separating the right wound from the anal region was kept in an aseptic condition by means of chloramine-T and its sides grew together without the need of counter-incision.



TEXT-FIG. 7. Experiment 7. Case 548. Preservation of asepsis of two deep wounds in the region of the buttocks by the use of chloramine-T, 10 parts per 1,000. The solid line indicates the right wound; the broken line, the left wound.



TEXT-FIG. 8. Experiment 8. Case 516. Preservation of asepsis in an open fracture by means of chloramine paste, 7 and 10 parts per 1,000.

July 10. Closing of the left wound.

Aug. 6. The right wound is sutured. The curve indicated that the wounds remained aseptic during the treatment.

Experiment 8. Case 516. Maintenance of an Osseous Cavity in an Aseptic Condition with Chloramine-T, 7 and 10 Parts per 1,000.—May 17, 1916. Fracture of the upper part of the left tibia. Local sterilization with sodium hypochlorite.

June 4. At the level of the fracture there is a cavity the size of a small egg, containing grayish secretions. The opening of the cavity is at the base of a wound situated on the inner surface of the leg. The wound and cavity are surgically sterile. They are filled with chloramine-T paste, 5 parts per 1,000.

June 6. The paste has not been absorbed. Between the paste and the surface of the cavity is a fluid, transparent substance. Slight reinfection; 4 bacteria per field. Chloramine dressing, 7 parts per 1,000.

June 7. Wound is sterile (Text-fig. 8).

June 13. Wound is sterile. After the osseous cavity has been filled with chloramine paste a portion of the wound is sutured, but the loss of cutaneous substance effected at the time the wound occurred prevents its complete closure. The open part of the wound is covered with chloramine paste, 7 parts per 1,000. The wound remains aseptic.

July 10. Chloramine paste is removed from the osseous cavity. The walls of the cavity are seen to be covered with granulations. Without removing the paste which remains in the cavity, the latter is filled with chloramine paste, 10 parts per 1,000. The osseous cavity has remained completely aseptic. The slight reinfections shown by the curve exist only at the edges of the wound.

Aug. 22. The cavity has almost filled and is covered with epithelium.

Experiment 9. Case 625. Preservation of Asepsis in Five Flesh Wounds with Chloramine-T, 10 Parts per 1,000.—Numerous wounds in the left leg produced by bursting shells.

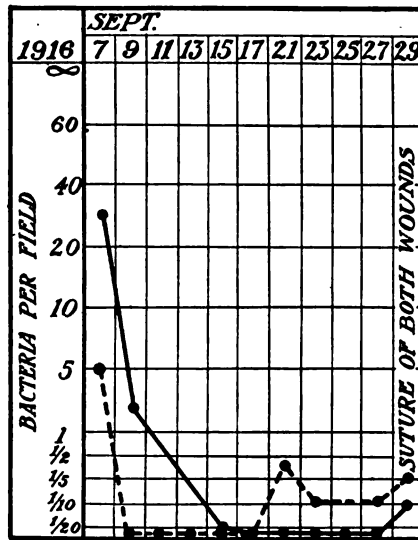
Sept. 5, 1916. Patient admitted to the hospital. The wounds are covered with pus and are treated with sodium hypochlorite.

Sept. 11. The pus has disappeared and the wounds are sterile (Text-fig. 9). Chloramine dressing, 10 parts per 1,000.

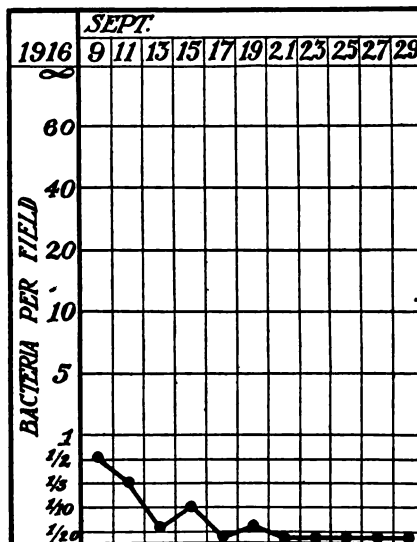
Sept. 13. Suture of three of the wounds; the other two are dressed with chloramine-T. They remain sterile through Sept. 29, when they are sutured.

Experiment 10. Case 590. Preservation of Asepsis in a Flesh Wound with Chloramine-T, 10 Parts per 1,000.—Wounds in the right thigh, accompanied by femoral fracture, produced by bursting shells. Treated with sodium hypochlorite.

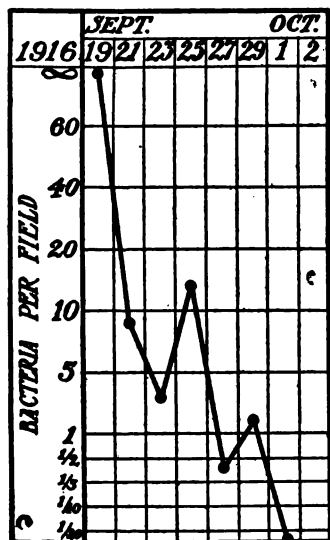
Sept. 11, 1916. The anterior wound is surgically aseptic (Text-fig. 10). Chloramine dressing. The wound is separate from the site of the fracture and remains sterile through Sept. 29 under chloramine dressing.



TEXT-FIG. 9. Experiment 9. Case 625. Preservation of asepsis of flesh wounds with chloramine-T, 10 parts per 1,000. The solid line indicates the posterior wound; the broken line, the anterior wound.



TEXT-FIG. 10. Experiment 10. Case 590. Preservation of sterility in a wound accompanied by femoral fracture with chloramine-T, 10 parts per 1,000.



TEXT-FIG. 11. Experiment 11. Case 620. Sterilization of a slightly infected wound with chloramine-T, 10 parts per 1,000.

Experiment 11. Case 620. Sterilization of a Slightly Infected Flesh Wound with Chloramine-T, 10 Parts per 1,000.—Numerous shell wounds. A large wound in the left buttock is treated with sodium hypochlorite. It is gradually cleansed.

Sept. 25, 1916. Wound contains 10 to 20 bacteria per field (Text-fig. 11). There are practically no secretions. Chloramine dressing, 10 parts per 1,000.

Sept. 27. Wound is surgically aseptic; same dressing.

Oct. 1. The bacteria have completely disappeared.

Experiment 12. Case 634. Reinfection Followed by Sterilization of Two Flesh Wounds with Chloramine-T, 10 Parts per 1,000.—Numerous shell wounds on the forehead, shoulder, and elbow. Disinfection of wounds with Dakin's hypochlorite solution.

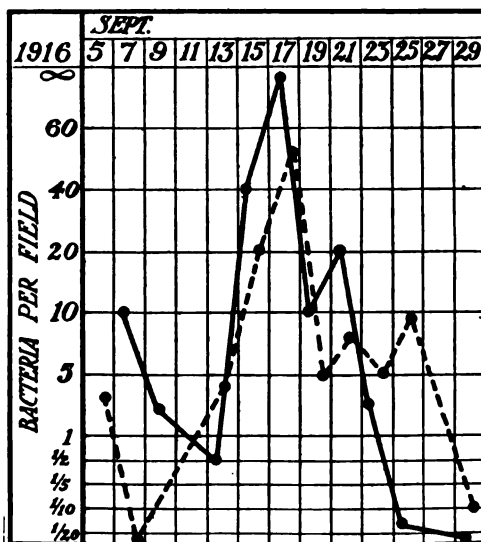
Sept. 11, 1916. The wounds on the forehead and left shoulder are almost sterile (Text-fig. 12). Application of chloramine paste and gauze dressing. The patient, who has also a severe fracture of the elbow and several other wounds, is very restless and the surface dressings become displaced.

Sept. 15 and 17. Reinfection from the skin. Considerable increase in the number of bacteria.

Sept. 17. Washing with neutral sodium oleate. The chloramine dressing is firmly fixed in place.

Sept. 19 and 21. Rapid diminution in the number of bacteria. Same dressing.

Sept. 29. Both wounds are sterile.



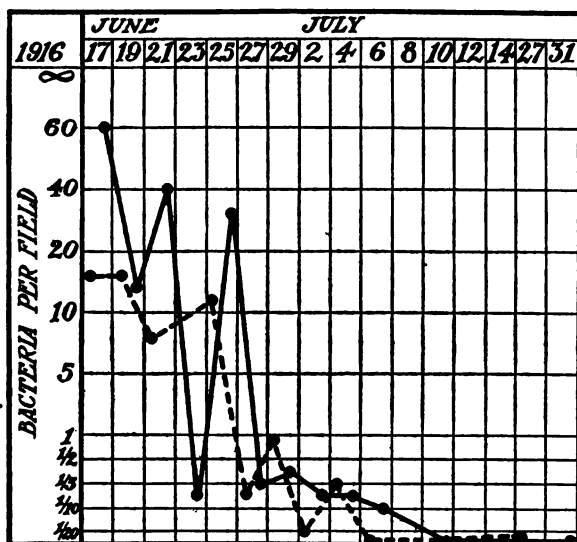
TEXT-FIG. 12. Experiment 12. Case 634. Reinfection of cutaneous origin, caused by shifting of dressing, and subsequent sterilization with 10 parts per 1,000 of chloramine-T. The solid line indicates the wound on the forehead; the broken line, the wound in the left shoulder.

The six experiments given above were selected from a series of sterile or slightly infected wounds treated with chloramine-T paste, 10 parts per 1,000. In every case the chloramine-T either maintained or produced the sterility of wounds infected from the skin. Nevertheless, this result can only be obtained if the dressings are made according to the technique described above. In Experiment 12, the dressings had not been firmly fixed at the surface of the wounds. The gauze shifted slightly and a cutaneous reinfection occurred. But sterilization was reestablished as soon as the appropriate technique was applied.

Sterilization of a Wound with Chloramine-T, 10 Parts per 1,000.

The above experiments and many others having shown that chloramine-T, 10 parts per 1,000, preserves a wound in an aseptic condition for several days and weeks, we next endeavored to ascertain whether it would also effect the sterilization of an infected wound.

The first of these experiments was made with surface wounds showing no necrotic tissue and slight infection. Later they were applied to wounds accompanied by a fracture.



TEXT-FIG. 13. Experiment 13. Case 519. Sterilization of two surface wounds of the left thigh with chloramine-T, 10 parts per 1,000. The solid line indicates the inside wound; the broken line, the posterior wound.

Experiment 13. Case 519. Sterilization of Two Flesh Wounds with Chloramine-T, 10 Parts per 1,000.—Two wounds of the thigh sterilized with sodium hypochlorite; subsequent reinfection. The wound on the inner side measures 24 sq. cm.; that towards the back, 19 sq. cm.

June 25, 1916. Both wounds are covered with smooth red granulations. No necrotic tissue; small amount of secretion; slight infection. Cocci, diplococci, and bacilli appear on the inside wound in the proportion of 30 per field, and 12 per field in the posterior wound (Text-fig. 13). Application of chloramine paste, 7 parts per 1,000.

June 26. Inside wound shows cocci, diplococci, and streptococci averaging 10 to 15 per field. In the posterior wound there are masses of cocci—6 to 8 per field. Washing with neutral sodium oleate; dressing with chloramine paste, 7 parts per 1,000.

June 27. Inside wound contains 1 coccus in 5 fields; posterior wound contains 1 coccus in every 7 fields.

June 30. Chloramine dressing, 10 parts per 1,000.

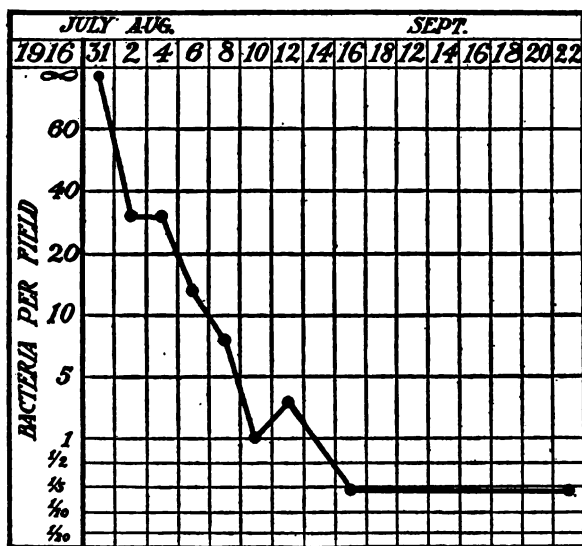
July 1. Inner wound shows 1 coccus in 8 fields; posterior wound shows 1 coccus in 8 fields. Same dressing.

July 4. Few bacteria. From this time on the wounds remain sterile.

July 27. The posterior wound has healed.

July 31. The inside wound has healed.

This experiment shows that a wound covered with smooth granulations, devoid of necrotic tissue, with a slight quantity of secretion, and a varied but sparse bacterial flora, may be completely sterilized with chloramine-T paste. The experiment was repeated with a large number of wounds and always produced identical results. Thereafter the same treatment was applied to more heavily infected wounds which still contained some necrotic tissue.



TEXT-FIG. 14. Experiment 14. Case 591. Sterilization of a seton wound in the sole of the foot with chloramine-T, 10 parts per 1,000.

Experiment 14. Case 591. Sterilization of a Seton Wound in the Sole of the Foot with Chloramine-T, 10 Parts per 1,000.—Seton wound in the sole of the foot produced by a fragment of a torpedo on July 24.

July 31, 1916. The wound is very much inflamed and infected. Large number of bacteria (Text-fig. 14). Treatment with Dakin's hypochlorite solution.

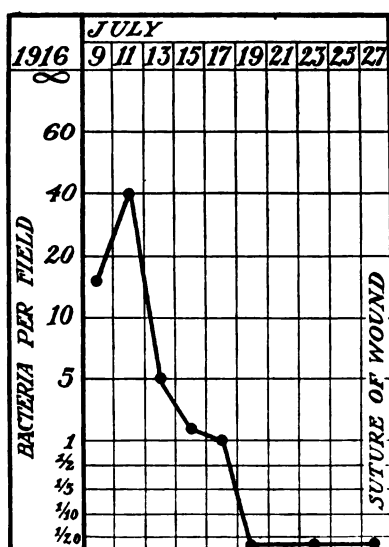
Aug. 3. Sole of the foot still infected. At the surface a small amount of necrotic tissue remains. Radiological examination of the wall of the wound shows the presence of nine minute fragments which cannot be removed owing to their small size. Injection of chloramine paste into the channel and chloramine dressing, 10 parts per 1,000.

Aug. 4. The wound still contains 30 bacteria per field.

Aug. 6 and 8. The wound is becoming sterile.

Aug. 10. The wound now contains only 1 bacterium per field.

Aug. 16. The wound has become surgically sterile. It remains in this condition through Sept. 22, when complete sterilization is effected.



TEXT-FIG. 15. Experiment 15. Case 562. Sterilization with chloramine-T, 10 parts per 1,000, of a slightly infected wound in the right buttock, accompanied by fracture of the ilium.

Experiment 15. Case 562. Sterilization of a Wound Accompanied by Fracture with Chloramine-T, 10 Parts per 1,000.—July 4, 1916. Large shell wound in the upper part of the right buttock and fracture of the ilium. Wound is first treated with Dakin's hypochlorite solution.

July 9. The surface of the wound is cleansed. The secretions average 15 bacteria per field (Text-fig. 15). Dressing with chloramine paste, 10 parts per 1,000.

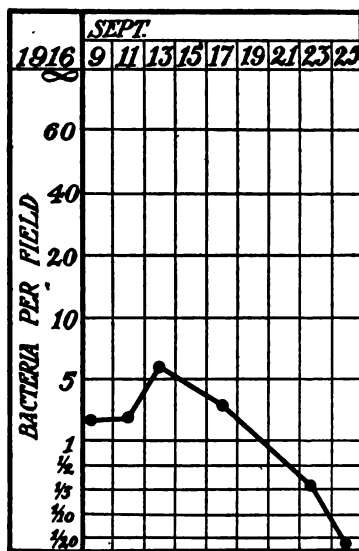
July 10. Wound is covered throughout with red granulations; only at isolated points is it still grayish in color. Chloramine dressing, 10 parts per 1,000.

July 13. 5 bacteria per field.

July 17. The grayish spots have disappeared. About 1 bacterium per field.

July 20. Wound is sterile.

July 27. Wound is sutured.



TEXT-FIG. 16. Experiment 16. Case 646. Sterilization with 10 parts per 1,000 of chloramine-T of a deep, slightly infected wound.

Experiment 16. Case 646. Sterilization of a Slightly Infected Flesh Wound with Chloramine-T, 10 Parts per 1,000.—Sept. 8, 1916. Numerous suppurating and severely infected wounds on the left leg. Two of the wounds are treated for 48 hours with Dakin's hypochlorite solution.

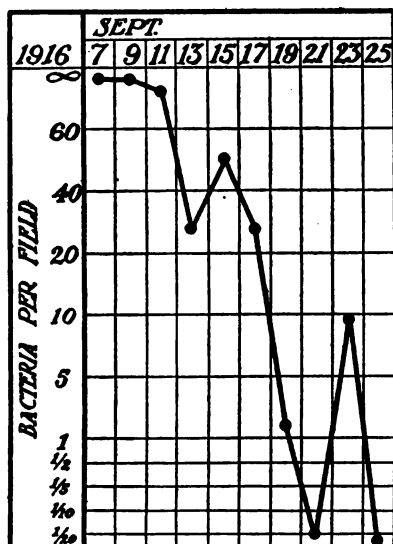
Sept. 11. The inside wound is still infected (Text-fig. 16). It is deep, and the calf is still inflamed. Chloramine dressing, 10 parts per 1,000.

Sept. 23. Wound is surgically sterile.

Experiment 17. Case 441. Sterilization of a Small, Severely Infected Wound with Chloramine-T, 10 Parts per 1,000.—Sept. 4, 1916. Removal by a bursting shell of the fourth and fifth fingers of the left hand, and of the greater part of the fifth metacarpal.

Sept. 6. Suppurating wound, severely infected and painful, containing necrotic tissue and large numbers of bacteria. Treatment for 5 days with Dakin's hypochlorite solution.

Sept. 11. The wound still contains large numbers of bacteria in the necrotic parts (Text-fig. 17). Red granulations have appeared. Dressing with chloramine paste, 10 parts per 1,000.



TEXT-FIG. 17. Experiment 17. Case 441. Sterilization of a severely infected wound containing a small quantity of necrotic tissue with chloramine-T, 10 parts per 1,000.

Sept. 13. The wound contains 30 bacteria per field. Same dressing.

Sept. 19. Wound is almost sterile; contains 1 bacterium per field.

Sept. 21. A few bacteria are found on a small fragment of necrotic aponeurosis.

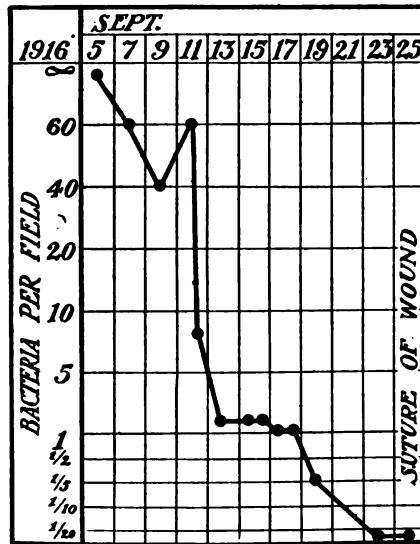
Sept. 25. Wound is sterile.

Experiment 18. Case 626. Sterilization with Chloramine-T, 10 and 15 Parts per 1,000, of a Large, Severely Infected Wound, Accompanied by Fracture.—Extensive wound, more than half the thickness of the forearm in depth, with fracture of the radius and cubitus.

Sept. 5, 1916. The wound, which is 9 days old, appears grayish in color. The muscular and osseous surfaces have been evened, but they are still covered with necrotic tissue and blue pus. The limb is swollen and painful. Chloramine dressing, 10 parts per 1,000.

Sept. 7. The blue pus has almost completely disappeared. At the surface and bottom of the wound some necrotic tissue remains. Application of chloramine paste, 15 parts per 1,000, at the level of the osseous extremities. The rest of the wound is covered with chloramine-T, 10 parts per 1,000.

Sept. 13. The wound now contains 1 bacterium per field (Text-fig. 18). The granulations of the section of the wound treated with chloramine paste, 15 parts per 1,000, have assumed a deep red color. The necrotic tissue has completely disappeared.



TEXT-FIG. 18. Experiment 18. Case 626. Sterilization with 10 and 15 parts per 1,000 of chloramine-T of a wide, severely infected wound of the forearm, accompanied by fracture of the upper part of both bones, and with pyocyanic infection and necrotic tissue.

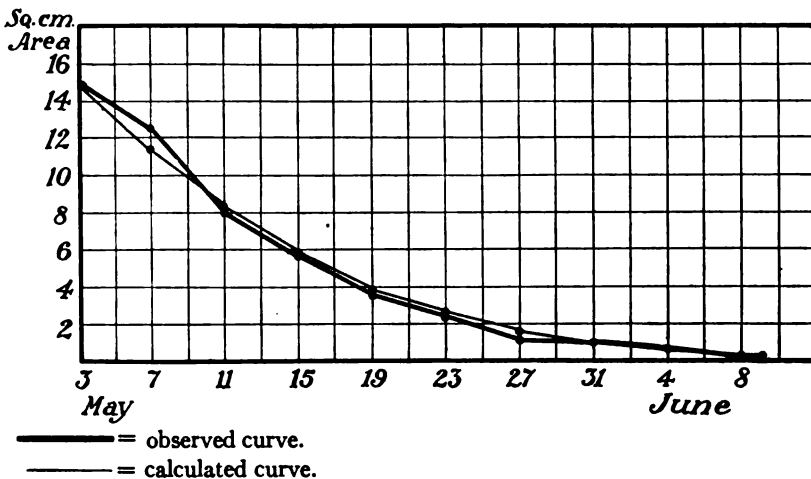
- Sept. 19. Wound is surgically sterile.
 Sept. 23. The bacteria have disappeared from the films.
 Sept. 25. Wound is sutured.

The wounds in the above experiments were in different stages of infection. Some were simple flesh wounds unaccompanied by necrotic tissue and with but slight suppuration. Others showed necrotic tissue and greater suppuration, while in a third group cases of severe local infection were accompanied by fracture. The wound in Experiment 18 was complicated by fracture of the radius and ulna and showed abundant suppuration. Moreover, it contained a large number of *pyocyaneus* bacilli. All these wounds were easily sterilized with chloramine-T paste, 10 or 15 parts per 1,000. In 48 hours the blue pus had almost completely disappeared from Wound 18. But sterilization took place more slowly than would have been the case if instillations of sodium hypochlorite had been used. These results were obtained with a paste containing 10 to 15 parts per 1,000 of

chloramine-T. The 15 parts per 1,000 of chloramine-T caused slight irritation to the skin and congested the granulations. Apparently the concentration of chloramine-T must not exceed 10 parts per 1,000.

Effect of Chloramine Paste on the Rate of Cicatrization.

In the following experiments an attempt was made to determine whether chloramine-T, when used in a concentration of 4 and of 10 parts per 1,000, has any effect upon the process of cicatrization. The chloramine paste was applied to wounds whose surface was measured every 4 days, and whose healing curve had been calculated according to the formula of du Noüy. By comparing the curves as calculated and observed we were able to determine whether the action of the chloramine paste retarded the process of cicatrization. Experiments were made with surface wounds treated with chloramine-T in a concentration of 4 and 10 parts per 1,000. The results were approximately identical in all cases. Only two typical experiments will be described.



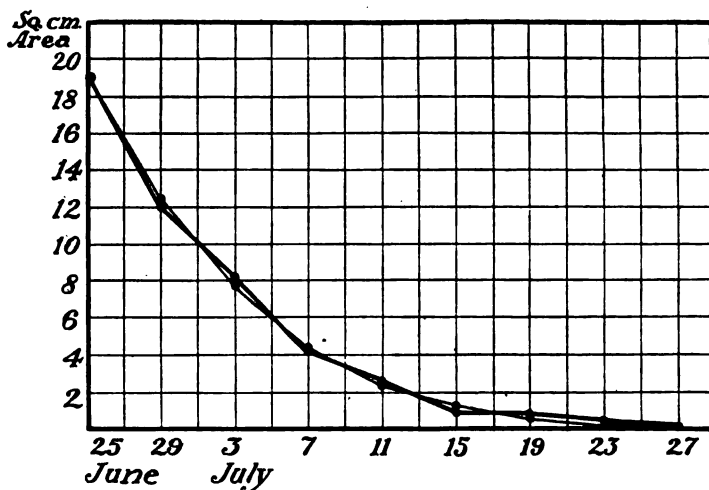
TEXT-FIG. 19. Experiment 19. Case 450. Effect of 4 parts per 1,000 of chloramine upon the rate of cicatrization of a wound. Application of chloramine paste on May 3 to 13.

Experiment 19. Case 450. Effect of Chloramine-T. 4 Parts per 1,000, on the Cicatrization of an Aseptic Wound.—Granulous wound.

May 3, 1916. Wound almost sterile and contains only a few bacteria. The surface measures 15 sq. cm. The cicatrization curve, calculated according to the formula of du Nott, shows that the healing process should be accomplished on June 8. Chloramine dressing, 4 parts per 1,000.

May 7. The wound is sterile; same dressing. From this time on the cicatrization curve descended and caught up with the calculated curve (Text-fig. 19).

From May 11 to 13, at which time wax was substituted for the chloramine-T, the curve followed the calculated curve.



TEXT-FIG. 20. Experiment 20. Case 519. Effect of chloramine-T, 7 and 10 parts per 1,000, upon the rate of cicatrization of a wound. The wound was dressed from June 27 to 30 with chloramine-T, 7 parts per 1,000, and from June 30 to July 27 with chloramine-T, 10 parts per 1,000.

Experiment 20. Case 519. Effect of Chloramine-T, 7 and 10 Parts per 1,000, upon the Cicatrization of an Aseptic Wound.—Granulating wound at the back of the thigh.

June 25, 1916. Superficial measurement, 19 sq. cm. The secretions contain 10 bacteria per field. The course of cicatrization is calculated according to the formula of du Nott.

June 27. The wound is surgically aseptic. Chloramine dressing, 7 parts per 1,000, is used until June 30.

June 30 to July 27. Chloramine dressing, 10 parts per 1,000. The curve coincided almost exactly with the calculated curve; nevertheless, after July 19 slight retardation of the healing process apparently occurred (Text-fig. 20).

The above experiments, as well as others that are analogous, show that sodium stearate containing from 4 to 10 parts per 1,000 of chloramine-T does not retard the process of cicatrization to an appreciable extent. In Experiment 20 during the first 25 days the observed curve coincided with the calculated curve, after which the rate of healing became slightly reduced.

SUMMARY.

Sodium stearate has no effect upon the bacteriological condition of a wound, but the addition of 4 parts per 1,000 of chloramine-T renders it antiseptic. Experiment 1 enabled us to compare the action of sodium stearate alone with that of sodium stearate containing 4 parts per 1,000 of chloramine-T. Wounds which had been previously sterilized could be maintained in an aseptic condition by 4 parts per 1,000 of chloramine-T, although in some cases reinfection occurred (Experiments 2, 3, and 5). For this reason the concentration of chloramine-T was increased.

Surface wounds, deep-seated wounds, and osseous cavities, which had previously been either completely or almost completely sterilized, could be maintained for days and even weeks in a condition of surgical asepsis by the use of a paste containing 7 and 10 parts per 1,000 of chloramine-T. Experiments 7, 8, 9, 10, and 12 are examples of this. Slightly infected wounds (Experiments 9, 11, and 12) were sterilized in the same manner.

Next, it was attempted to sterilize wounds which were suppurating and more or less infected, and in some cases accompanied by fracture. This attempt was probably successful because the wounds used for the experiments showed but slight quantities of secretions and only a shallow layer of necrotic tissue. It is useless to attempt to sterilize severely infected wounds with a paste, for the volume of chloramine-T that can be applied is too limited. A large volume of an active substance is required to sterilize a wound which secretes great quantities of pus, for owing, on the one hand, to the dilution of this substance with the secretions, and, on the other, to its combination with the proteins contained in the pus, the concentration of the antiseptic is rapidly diminished. For these reasons it is essential that the antiseptic solu-

tion should be constantly renewed, so that the concentration may be sufficiently strong to effect the destruction of the bacteria. Therefore, the chloramine-T paste cannot sterilize a severely infected wound.

The concentration of the active substance contained in a paste must at the same time be sufficiently weak to be innocuous to the tissues. We have seen that it should not exceed 15 parts per 1,000. Thus, it is evident that if the secretions from the wounds are abundant, the substance could exert its action upon the microorganisms for the space of only a few hours. For this reason the chloramine paste should only be applied under the conditions specified in our experiments; that is, in connection with moderately infected wounds which have been carefully washed with sodium oleate, and possess but slight quantities of secretion. Under these conditions, the chloramine paste effects the complete disappearance of the bacteria and maintains the sterility thus secured for as long a time as may be wished. If the technique followed in the dressing is not exactly as above described, reinfection will occur. If applied in this manner the chloramine paste is not injurious to the tissues, for the cicatrization curves of the wounds thus treated show but slight modification from the calculated curves.

Chloramine paste makes it possible, therefore, to keep wounds sufficiently free from microorganisms so that the effect of substances which are believed to influence cicatrization can be studied.

CONCLUSIONS.

1. Under the conditions of our experiments chloramine paste maintains the asepsis of a wound already sterile, and sterilizes an infected wound.
2. Under the same conditions chloramine paste causes no apparent modification of the cicatrization curve of an aseptic wound.

CICATRIZATION OF WOUNDS.

IX. INFLUENCE ON THE HEALING OF WOUNDS OF VARIATIONS IN THE OSMOTIC TENSION OF THE DRESSING.

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In previous articles it has been shown that the curve representing the process of cicatrization of an aseptic wound is geometric,^{1, 2} and can be calculated by the formula of du Noüy. The comparison of the calculated and observed curves alters the study of the effect upon cicatrization of a substance applied to the surface of the wound. If the daily decrease of the area of a wound is known, and if the wound is maintained in a condition of surgical asepsis, the modifications in the rate of the healing process can be attributed to the special action of the substance applied, and the extent of this action can be accurately measured.

Up to the present it has not been known to what degree the rate of cicatrization of a wound can be affected by the dressing. Surgeons have studied the influence of so called healing substances when applied to wounds, but no precise conclusion has resulted from these observations. No method existed of measuring exactly the surface of a wound and of calculating with any degree of accuracy the rate of the healing process. On the other hand, the bacteriological condition of the wounds experimented upon was never taken into consideration. Such modifications of the rate of healing as were noticed might be equally attributable to the action of the substance employed upon the bacteria of the wound as to the tissues themselves.

¹ Carrel, A., and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

² du Noüy, P. L., *J. Exp. Med.*, 1916, xxiv, 451.

The following experiments were undertaken to ascertain whether modifications in the osmotic tension of the dressing exert an influence on the rate of repair.

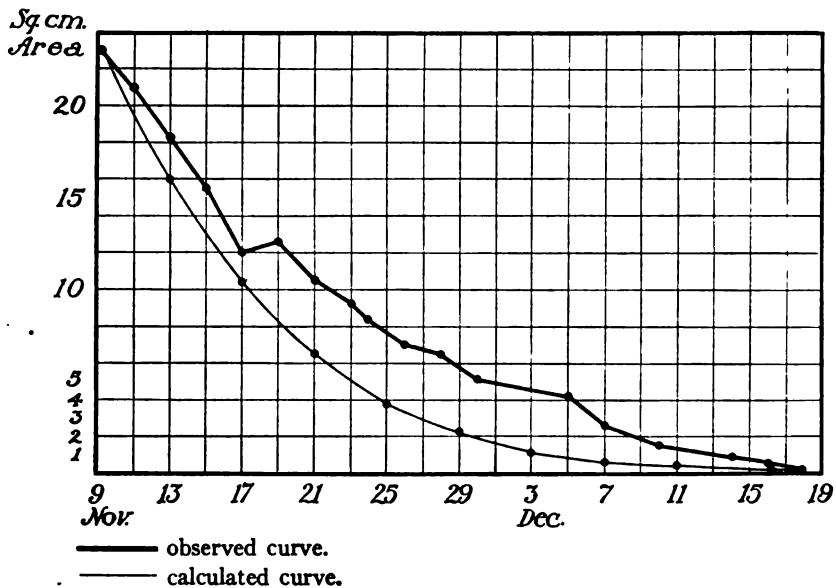
EXPERIMENTAL.

Surface wounds already covered with granulating tissue were selected. The normal rate of cicatrization of the wound was first obtained by sterilization with Dakin's hypochlorite solution or Daufresne's chloramine paste. The experiment was started as soon as the regular progress of the observed curve, as compared with the curve calculated according to du Noüy's formula, was established. The measurements of the wound and the plotting of the curve were made according to the technique previously described. Distilled water or hypertonic solution was applied to the surface of the wound by means of small perforated rubber tubes bound at the extremities and enveloped by a small cylindrical pad of Turkish toweling. The length of the pad varied from 4 to 8 cm., according to the size of the wound. Four threads placed transversely across the pad extended over the wound and were attached to the skin by means of small adhesive patches in such a manner as to keep the flushing tube in a fixed position over the granulations. The sterile liquid was contained in a flask placed about 1 meter above the patient's mattress, and reached the flushing tube by means of a Murphy drop tube. As a rule, in these experiments about 125 gm. of fluid flowed out per hour. Another technique was also used to bring into contact the surface of the wound and the distilled water or hypertonic solution. Agar cakes containing distilled water or hypertonic sodium chloride solution were applied to the surface of the wound. These constantly gave up their fluid contents, and were left at the surface of the wound, according to the nature of the experiment, from 6 to 12 hours and even 24 hours per day. In order to prevent immediate reinfection, the wound was flushed from four to six times in 24 hours with Dakin's solution. Another method consisted in applying for several hours every day a dressing composed of sodium stearate and chloramine paste. Chloramine paste, 10 parts per 1,000 was generally used, because reinfection takes place under 4 parts per 1,000. The wound

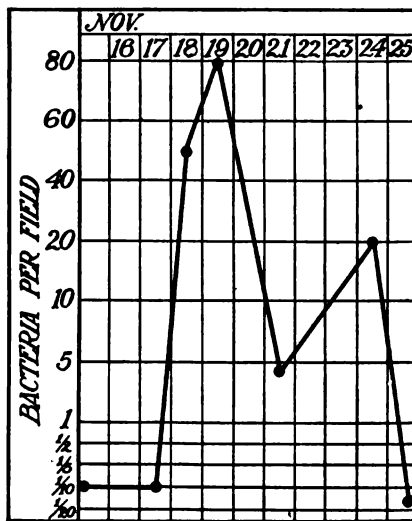
was examined bacteriologically every day. As soon as reinfection occurred the experiment was interrupted and the wound thoroughly sterilized. Every 4 days the surface of the wound was measured and the curve was plotted and compared with the calculated curve. The patients were kept in bed during the experiment and were under the supervision of a nurse day and night.

Influence of Distilled Water on the Rate of Healing of a Sterile Wound.

In the following experiments the wounds were flushed with distilled water from 2 to 4 hours every day.



Cicatrization curve.



Bacteriological curve.

TEXT-FIG. 1. Experiment 1. Case 646.

Experiment 1 (Text-fig. 1).—Case 646, age 25 years.

Nov. 17, 1916. Sterile wound in the calf, 12 sq. cm. in area. The wound is surgically aseptic. Flushing with distilled water for 2 hours. Then flushing with 30 per cent hypertonic solution for 2 hours. The dressing was kept in place until the following morning.

Nov. 18. The appearance of the wound has not changed. Surface area 12.1 sq. cm. 30 to 50 bacteria per field. Same dressing. Flushing with distilled water for 2 hours. Flushing with a hypertonic solution for 2 hours.

Nov. 19. The appearance of the wound has remained the same. Surface area 12.6 sq. cm. 50 to 100 bacteria per field. Dressing with chloramine paste, 10 parts per 1,000.

Nov. 20. Same dressing. Surface area 11.1 sq. cm.

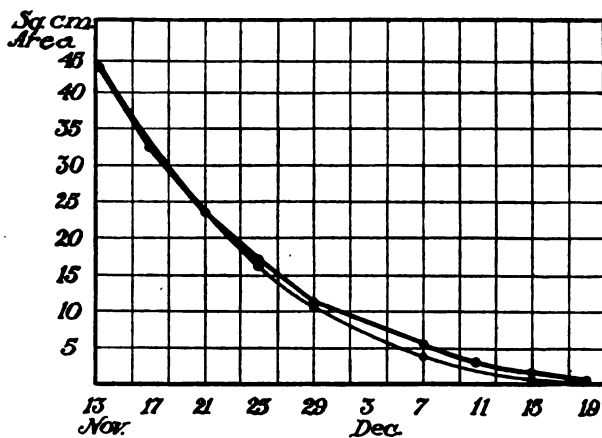
Nov. 21. Chloramine dressing, 4 parts per 1,000. Surface area 10.5 sq. cm. 4 bacteria per field.

Nov. 22. Flushing with distilled water for 4 hours. Chloramine dressing, 4 parts per 1,000 for 20 hours.

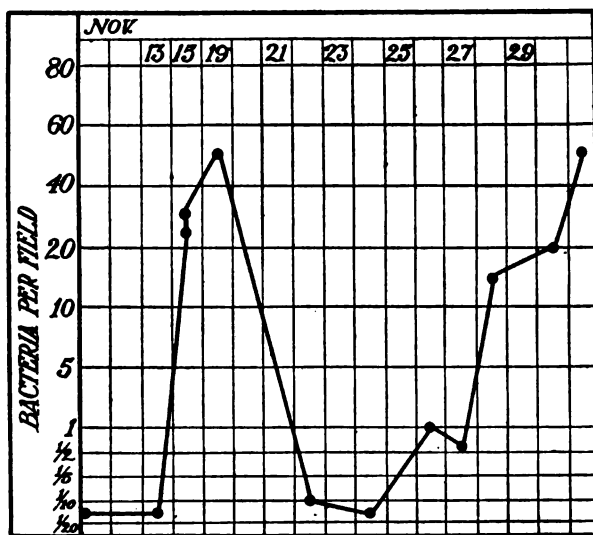
Nov. 23. Granulations smaller. Surface area 9.2 sq. cm. Flushing with distilled water for 4 hours. Chloramine dressing, 4 parts per 1,000.

Nov. 24. Same appearance. Surface area 8.4 sq. cm. 18 to 20 bacteria per field. Numerous cocci. Flushing with distilled water for 8 hours. Chloramine dressing, 10 parts per 1,000.

Nov. 25. 1 bacterium in 15 fields.



Cicatrization curve.



Bacteriological curve.

TEXT-FIG. 2. Experiment 2. Case 694.

Experiment 2 (Text-fig. 2).—Case 694, age 20 years. Sterile wound 33.5 sq. cm. in area.

Nov. 17, 1916. Flushing with distilled water for 2 hours, followed by 2 hours' flushing with 30 per cent hypertonic sodium chloride solution. The dressing was not renewed until the following morning.

Nov. 18. The wound appears the same. Surface area 30.4 sq. cm. 20 to 30 bacteria per field. Flushing with distilled water for 2 hours and hypertonic solution for 2 hours.

Nov. 19. Same appearance. Owing to the infection, the rate of cicatrization has diminished. The surface area is 29.6 sq. cm. 55 bacteria per field. Chloramine dressing, 10 parts per 1,000.

Nov. 20. Same appearance. The curve has caught up with the normal curve. Surface area 26 sq. cm. 20 bacteria per field. Chloramine paste, 10 parts per 1,000.

Nov. 21. Same appearance. Surface area 23.6 sq. cm. 4 bacteria per field. Chloramine dressing, 4 parts per 1,000.

Nov. 22. Chloramine dressing, 4 parts per 1,000. 1 bacterium per 8 or 10 fields.

Nov. 23. Same treatment. Surface area 17.3 sq. cm. The wound is sterile and the experiment with distilled water alone can be resumed.

Nov. 24. Flushing with distilled water for 8 hours. Dressing with chloramine paste, 4 parts per 1,000. 1 bacterium in 10 to 15 fields.

Nov. 25. Flushing with distilled water for 8 hours.

Nov. 26. Flushing with distilled water for 24 hours. In order to keep the wound sterile, an injection of Dakin's solution is made every 6 hours. Surface area 14.6 sq. cm.; calculated area 14 sq. cm. 1 bacterium per field.

Nov. 27. Flushing with distilled water for 24 hours. 1 bacterium in 3 fields.

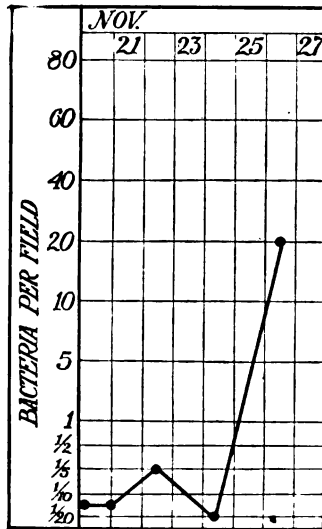
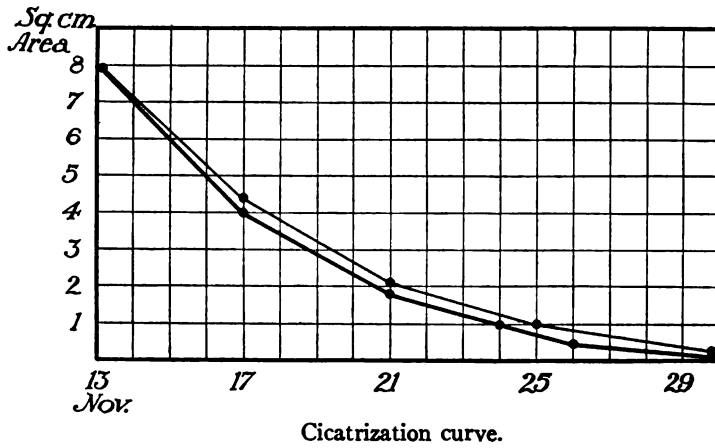
Nov. 28. Flushing with distilled water for 24 hours. Observed area 12 sq. cm. No apparent change. Calculated area 11.8 sq. cm. 10 to 15 bacteria per field.

Nov. 29. Flushing with distilled water for 24 hours.

Nov. 30. Retardation of the healing process. Observed area 11 sq. cm.; calculated area 9 sq. cm. 15 to 20 bacteria per field. Flushing with distilled water for 24 hours.

Dec. 1. Experiment interrupted on account of infection. Application of compresses soaked in Dakin's solution; renewed six times in 24 hours.

Dec. 2. Same dressing. Innumerable small bacilli.



TEXT-FIG. 3. Experiment 3. Case 694.

Experiment 3 (Text-fig. 3).—Case 694, age 20 years.

Nov. 21, 1916. Sterile wound of the wrist, measuring 1.8 sq. cm. Since Nov. 13 the process of repair has been regular. Flushing with distilled water for 2 hours, followed as in the previous experiment by application of chloramine paste, 4 parts per 1,000, in order to prevent infection.

Nov. 22. Flushing with distilled water for 4 hours, followed by chloramine dressing. Observed surface 1.5 sq. cm.; calculated surface 1.8 sq. cm. 1 bacterium in 4 fields.

Nov. 23. Flushing with distilled water for 6 hours. Dressing with chloramine paste.

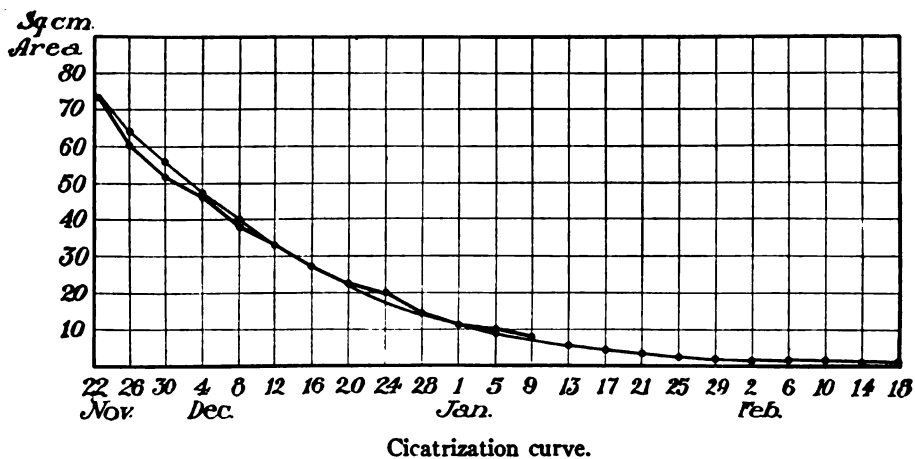
Nov. 24. Flushing with distilled water for 8 hours. Dressing with chloramine paste. 1 bacterium in 15 to 20 fields.

Nov. 25. Scale formed on the wound. Dry dressing.

Nov. 26. Scale is removed. Area of the wound is 0.37 sq. cm.; calculated surface 0.7 sq. cm. 15 to 20 bacteria per field. Dressing with chloramine paste.

Influence of Hypertonic Sodium Chloride Solution on the Rate of Healing of a Sterile Wound.

In the following experiments the wounds were flushed with hypertonic sodium chloride solution.



TEXT-FIG. 4. Experiment 4. Case 639.

Experiment 4 (Text-fig. 4).—Case 639, age 36 years.

Dec. 1, 1916. The wound is practically sterile. 1 bacterium in 10 to 12 fields.

Dec. 2. Flushing with 40 per cent sodium chloride solution for 6 hours.

Four injections with Dakin's solution. Surface area of wound 48.5 sq. cm.

Dec. 4. Flushing with 40 per cent sodium chloride solution for 12 hours. Four injections of Dakin's solution. Observed surface 46.5 sq. cm.; calculated surface 47.3 sq. cm. 3 bacteria per field.

Dec. 5. Same treatment.

Dec. 6. Same treatment. 1 bacterium in 8 to 10 fields.

Dec. 7. Same treatment.

Dec. 8. Same treatment. Six injections of Dakin's solution, instead of four. Area 38.5 sq. cm.; calculated area 39.7 sq. cm.

Dec. 9. Same treatment.

Dec. 10. Flushing with 40 per cent sodium chloride solution for 24 hours. Six injections of Dakin's solution. 1 bacterium in 8 fields.

Dec. 11. Same treatment.

Dec. 12. Same treatment. Surface of wound 33 sq. cm.; calculated surface 33 sq. cm. 1 bacterium per field. The curve as observed has finally overtaken the calculated curve and they now coincide exactly.

Dec. 13. Same treatment. 20 to 30 bacteria per field. The experiment is interrupted on account of infection.

Dec. 14. Sterilization of the wound by twelve flushings with Ringer solution.

Dec. 15. Same treatment. 1 bacterium in 6 fields.

Dec. 16. The experiment is resumed. A cake of agar-agar made of 40 per cent sodium chloride solution is applied to the wound during 12 hours. During the night the wound is sterilized by six flushings with Dakin's solution. Observed area 27 sq. cm.; calculated area 27.1 sq. cm. Sterile. The cake is applied for 9 hours.

Dec. 17. Same treatment. 4 bacteria per field.

Dec. 18. Same treatment. Surface observed 24.3 sq. cm.; calculated surface 24.2 sq. cm.

Dec. 19. A few bacteria.

Dec. 24. The wound is sterile. Agar cakes containing 50 per cent sodium chloride solution are applied during the day, and chloramine paste during the night.

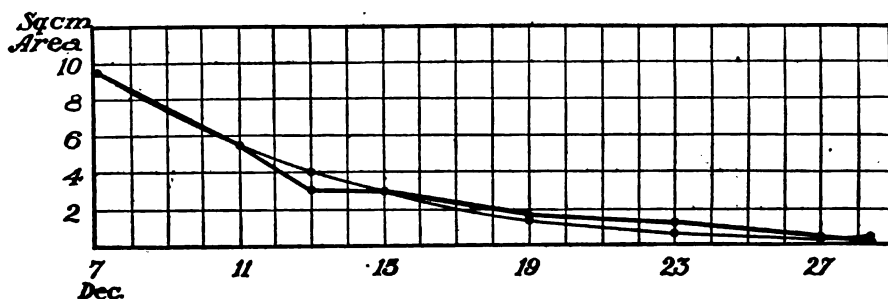
Dec. 28. The surface of the wound measures 14.2 sq. cm.; calculated surface 14.25 sq. cm. Same treatment.

Dec. 29. Same treatment.

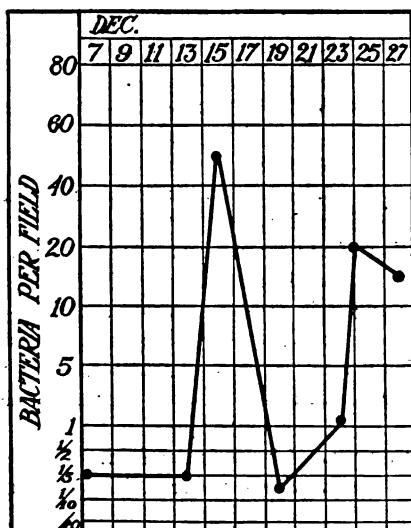
Dec. 30. Flushing with 80 per cent sodium chloride solution for 24 hours. Four flushings with Dakin's hypochlorite solution.

Jan. 1, 1917. Same treatment. Granulations congested. Small hemorrhages. Observed area 11 sq. cm.; calculated area 11.3 sq. cm.

Jan. 5. Same treatment with 80 per cent solution from Jan. 2 to 4. Observed area 10 sq. cm.; calculated area 9 sq. cm. 4 bacteria per field. The experiment is discontinued.



Cicatrizization curve.



Bacteriological curve.

TEXT-FIG. 5. Experiment 5. Case 715.

Experiment 5 (Text-fig. 5).—Case 715, age 23 years; Arabian. Leg wound.

Dec. 7, 1916. Surface of wound 9.5 sq. cm. Dressing with Dakin's solution.

Dec. 11. Surface of wound 5.5 sq. cm.; calculated area 5.5 sq. cm. Flushing with 50 per cent sodium chloride solution for 24 hours, interrupted with two injections of Dakin's solution. Same treatment until Dec. 15.

Dec. 15. 30 to 50 bacteria per field. Four flushings with Dakin's solution in 24 hours. Surface observed 2.85 sq. cm.; calculated area 2.9 sq. cm.

Dec. 18. Flushing with hypertonic solution is substituted by the application of a cake of agar-agar containing 40 per cent sodium chloride. During the night three injections with Dakin's solution are made.

Dec. 19. Surface observed 1.5 sq. cm.; surface calculated 1.35 sq. cm. The injections are replaced by applications of chloramine paste, 10 parts per 1,000, the agar cakes being kept in contact with the wound for 12 hours every day.

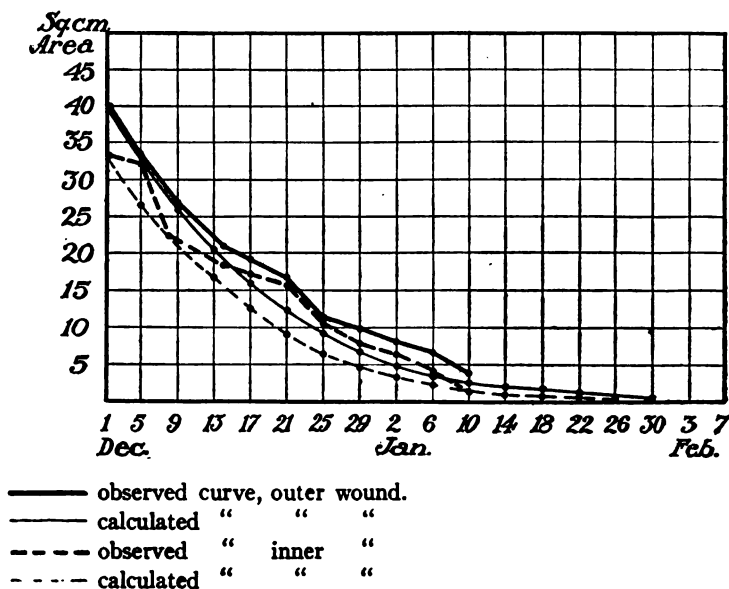
Dec. 23. Same treatment until Dec. 23. Observed area 1.1 sq. cm.; calculated area 0.6 sq. cm. The difference may be attributed to a slight infection. 2 bacteria per field.

Dec. 24. The 40 per cent agar cakes are replaced by a 50 per cent cake. The application of chloramine paste is continued during the night.

Dec. 27. Same treatment until Dec. 27, on which date it is calculated that healing will be effected. The wound, which is too small to be measured (less than 0.1 sq. cm.), is infected (10 to 15 bacteria per field), and this explains the retardation. Dressing with chloramine paste.

Dec. 29. The wound is completely healed.

Comparison of Distilled Water and of Hypertonic Sodium Chloride Solution on the Same Patient.



Cicatrization curve.

TEXT-FIG. 6. Experiment 6. Case 721.

Experiment 6 (Text-fig. 6).—Case 721. The patient has two wounds in the thigh, of about equal dimension, a small distance apart. The inner wound is flushed out with distilled water; the other with 40 per cent sodium chloride solution.

Dec. 3, 1916. Flushing of inner wound with distilled water for 12 hours. Four injections of Dakin's solution. Flushing of outer wound for 12 hours with 40 per cent sodium chloride. Four injections of Dakin's solution.

Dec. 4. 30 to 50 bacteria per field. Sterilization with Dakin's solution.

Dec. 8. Sterilization with Dakin's solution.

Dec. 12. Flushing for 24 hours per day under the same conditions described above, with six injections of Dakin's solution, continued until Dec. 16.

Dec. 17 and 18. Reinfection. Sterilization.

Dec. 20. Application to the external wound of an agar-agar cake, in the proportion of 40 per cent sodium chloride, and to the inner wound of a cake compounded with distilled water. 7 p.m. Dressing with chloramine paste. Same treatment until Dec. 27.

DISCUSSION AND SUMMARY.

In the study of the action of non-antiseptic substances on the rate of cicatrization, the chief obstacle encountered is the facility with which wounds become reinfected under an aseptic dressing. At the beginning of Experiment 1 the wound was sterile. It was subjected to flushing with distilled water for 2 hours, then to flushing with 30 per cent sodium chloride solution for another 2 hours. During that time no special precaution was taken to sterilize the wound and the dressing was left intact until the following morning. It was then found that the wound contained from 30 to 50 bacteria per field. The following day, after the wound had been subjected to the same treatment, the number of bacteria had increased to 50 and 100 per field, and as an immediate consequence the surface of the wound increased from 12 to 12.6 sq. cm. in 2 days. The wound was then dressed antiseptically and was found to be sterile 3 days later. Reinfection again took place the following day in spite of antiseptic dressing with chloramine paste 4 parts per 1,000, which was applied for 20 hours. In Experiment 2 similar results were observed. After 2 days of flushing with distilled water, the number of bacteria had increased to 50 per field. The wound was thereupon sterilized, but new reinfection ensued a few days later. Another wound on the same patient became reinfected under the same conditions after 1

day of sterile dressing. In none of the patients could the wounds be kept in a sterile condition throughout the whole experiment. It was impossible to maintain the sterility of a wound under aseptic dressing. Dakin's solution was therefore injected every 4 hours, or less often, according to the degree of infection, or chloramine paste was applied during the night. If there were 3 or 4 bacteria per field, the experiment was discontinued in order that the wound might be sterilized again. The cicatrization and bacteriological curves of Experiment 4 show that by the application of chloramine paste a wound may be maintained in an appropriately bacteriological condition for carrying out an experiment. Nevertheless, in spite of the antiseptic precautions taken, it was necessary to interrupt this experiment on two occasions, on December 13 to 15 and on December 18 to 22, in order that a complete sterilization of the wound might be effected. When the sterilization was performed as soon as the bacteria were discovered, little retardation occurred in the process of cicatrization. Moreover, the reinfection from the skin was often due to fine bacilli which have but mild retarding action on the rate of healing. The use of at least six flushings in 2 hours with Dakin's solution or of 12 hours' dressing with chloramine paste 10 parts per 1,000, was necessary to keep the wound in a condition of surgical asepsis.

The action of distilled water was studied in Experiments 1, 2, and 3. In Experiment 1 the wound was subjected to flushing with distilled water first for 2 hours, then 4 hours, and later for 8 hours per day. The wound was maintained in a condition of mild infection. No marked modification, either acceleration or retardation, was noted in the rate of repair during the period that the treatment was applied. From November 21 to 25 the wound was almost clean and the observed curve remained parallel to the calculated curve, showing that distilled water did not retard the rate of healing. In Experiment 2 the wound was subjected to uninterrupted flushing with distilled water, first for 2 and 8 hours, then for 24 hours. It was continued from November 24 to 30; *viz.*, for 112 hours out of 120, without the occurrence of any marked modification of the course of healing. The bacteriological curve showed that from November 22 to 27 inclusive the wound was kept aseptic. The slight retardation which occurred afterwards

was probably brought about by the infection. In Experiment 3 the wound was subjected to flushing with distilled water, first for 2, then for 4, 6, and 8 hours, a total of 20 hours in 4 days. From November 21 to 24 the wound remained surgically aseptic. No modification in the rate of healing occurred.

The action of the hypertonic sodium chloride solution was studied in a similar way. In Experiment 4 the wound was flushed at first with 40 per cent sodium chloride solution, from December 4 to 9 for 12 hours a day, and from December 10 to 13 for 24 hours a day, making a total of 144 hours out of 240 hours. At the end of this time the surface area of the wound coincided exactly with the calculated area. Owing to reinfection the experiment was suspended. From December 24 to 29 the wound was kept in contact with 50 per cent sodium chloride solution for 54 hours, and after December 30 flushing with 80 per cent solution for 24 hours a day was resorted to. The total amount of time involved in the above treatments was 174 hours with 40 per cent solution, 72 hours with 50 per cent solution, and 120 hours with 80 per cent solution. On January 1, the surface measured 11 sq. cm. and the calculated surface was 11.3 sq. cm. On January 5 the surface observed was 10 sq. cm. and the calculated surface was 9 sq. cm. It should be noticed that on January 5 the bacteria numbered 4 per field, which might account for the difference. In Experiment 5 the wound was flushed for 24 hours every day with 50 per cent sodium chloride solution from December 11 to 18, a total of 192 hours. From December 18 to 24 the wound was dressed with agar-agar cakes containing 40 per cent sodium chloride. The concentration was raised to 50 per cent from December 24 to 27. The cicatrization curve indicates only a slight retardation of the repair which can be attributed to infection when both cicatrization and infection curves are compared. The temporary acceleration on the 13th may have been due to the influence of the dressing, but as it did not occur again an experimental error is probably the cause of the change observed in the curve. In Experiment 6 two practically identical wounds at a distance of but a few centimeters from each other were located on the right thigh of Patient 721. The areas of the wounds were respectively 40 and 33 sq. cm. One of the wounds was flushed with distilled

water only. The other was subjected to the action of 40 per cent sodium chloride solution. From December 20 to 25 both wounds were in a condition of surgical asepsis. However, the cicatrization curves show that in spite of the difference of treatment the rate of healing was not modified.

The rate of healing of the wounds did not therefore apparently undergo any measurable modification under the influence of distilled water or hypertonic salt solution. It is well known that the osmotic changes of the medium have a marked influence on tissues deprived of circulation. But it seems that a tissue with normal circulation is protected by it against the changes of the osmotic pressure occurring at its surface. The above experiments show that apparently the conditions of the tissues of a wound are not modified by the changes of the osmotic pressure of the dressing. The beneficial effects of hypertonic sodium chloride solution on the sterilization of wounds and on the rate of healing recently described by various surgeons are possibly an illusion due to lack of precise technique.

CONCLUSIONS.

1. The flushing of an aseptic granulating wound with hypertonic sodium chloride solution or distilled water brings about an immediate reinfection.
2. Distilled water and hypertonic sodium chloride solution do not modify to a measurable extent the rate of healing of an aseptic wound.

APPLICATION DE LA MÉTHODE DE CARREL AUX FORMATIONS DE L'ARRIÈRE.

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(De l'Hôpital temporaire 21 et des laboratoires du Rockefeller Institute, Compiègne.)

La possibilité de stériliser par la méthode de Carrel les blessures fraîches de guerre est maintenant acquise. Dehelly et Dumas ont par leurs travaux, fait connaître les détails de la technique. Des ambulances du front¹, ouvertes à tous, enseignent la pratique de la désinfection des plaies et de leur fermeture.

Aujourd'hui une nouvelle question se pose. *Peut-on appliquer les mêmes procédés de traitement aux blessés évacués à l'intérieur?*

Il est de la plus haute importance de résoudre d'une manière précise ce problème. Personne ne nous contredira si nous affirmons qu'en raison des nécessités des évacuations une bonne majorité des blessés arrive infectée dans les hôpitaux de l'arrière². Si l'on prend la peine d'interroger les chirurgiens de ces formations, on reconnaît qu'ils sont presque unanimes à considérer comme difficile la stérilisation, suivie de fermeture, des plaies des parties molles, et comme tout à fait impossible celle des fractures, des lésions articulaires et des infections gangréneuses s'accompagnant de gaz.

Une telle opinion ne peut être acceptée que si elle s'appuie sur des expériences.

1. L'Ambulance de La Panne et l'Hôpital temporaire 21 à Compiègne.

2. Cotte, Traitement des fractures, *Lyon chirurgical*, septembre-octobre 1916, n° 5, p. 745, a recherché dans un hôpital de Paris le sort des fractures de cuisse d'une année en laissant de côté celles qui étaient arrivées amputées. Sur 21 blessés, 6 avaient été cicatrisés en 4 ou 5 mois; un était mort de septicémie au bout d'un mois; 3 avaient été évacués au bout de 8 à 10 mois, consolidés mais gardant une fistule; 11 en traitement depuis plus de 8 à 10 mois avaient dû être réopérés en raison de l'ostéomyélite qu'ils présentaient.

Les conditions de l'expérimentation.

Ces expériences nous les avons faites à l'hôpital temporaire 21 et dans les laboratoires de l'Institut Rockefeller à Compiègne, en nous plaçant dans des conditions de recrutement des blessés que nous croyons à l'abri de toute critique.

Nos sujets n'ont pas été choisis dans des ambulances. Ils ont été pris à E..., à partir du 5 septembre 1916, dans les trains descendant sur Paris. Nous les avons eus quelques heures seulement avant leur arrivée dans les hôpitaux auxquels on les destinait. Cette avance de quelques heures ne peut jouer aucun rôle dans les résultats, car la première série de 34 blessés à laquelle nous empruntons les éléments de ce travail, comprenait des lésions datant d'un minimum de 2 jours et d'un maximum de 46, en moyenne de 11. Nous étions donc bien dans des conditions identiques à celles des formations de l'arrière.

Les instructions données aux médecins envoyés par nous à E... avec nos voitures d'ambulance, étaient de rapporter, dans la mesure du possible, des fractures, ou des blessés ayant de la fièvre et portant des pansements odorants. Cette dernière recommandation avait sa valeur, car il nous arriva de trouver en une fois deux gangrènes gazeuses sur une série de six malades.

Les résultats.

Bien que le début de notre expérimentation ne remonte qu'à moins de deux mois¹, nous pouvons affirmer, dès maintenant, que les plaies anciennes se comportent d'une manière générale comme les plaies fraîches sous le rapport de la stérilisation et de la fermeture.

Nous disons "d'une manière générale", parce qu'au point de vue des suites il est nécessaire de faire un départ entre les blessés bien opérés et ceux qui le furent insuffisamment, ou même ne le furent pas du tout.—Les premiers évoluent comme des blessés frais. Les seconds ont besoin d'un acte opératoire préalable. Cela retarde le traitement; car, ainsi que nous le montrerons plus loin, il faut s'efforcer, surtout dans les fractures, de n'intervenir qu'à froid, comme on le ferait pour une appendicite ou une salpingite.

1. Cet article a été écrit fin octobre 1916.

Le peu de temps écoulé depuis le début de notre expérimentation, fait que plusieurs des blessés reçus depuis le 5 septembre 1916 sont encore en plein traitement. Cependant, pour les 34 premiers, le recul est suffisant pour que l'on puisse présenter un tableau des résultats. Voici ce tableau établi en conservant pour chaque cas l'ordre d'inscription sur les registres du service.

1° Gaï.....	Fracture de l'humérus.....	Suturé.
2° Gar.....	Fracture des 2 os de l'avant-bras.....	Suturé.
3° La.....	Fracture du fémur.....	Suturé.
4° Ma.....	Plaie pénétrante du genou.....	Suturé.
5° Lo.....	Très larges plaies des parties molles. Infection grave.....	Stérile.
6° Cr.....	Fracture de l'humérus et du coude.....	Stérile.
7° Ja.....	Fracture du radius et plaies multiples....	Suturé.
8° Th.....	Fracture de l'humérus.....	Suturé.
9° Me.....	Fracture de l'humérus.....	Suturé.
10° Fo.....	Fracture du tibia.....	Stérile.
11° Da.....	Fracture du tibia.....	Suturé.
12° Pi.....	Plaies des parties molles.....	Suturé.
13° Ca.....	Perforation de la tête humérale. Large plaie des parties molles.....	Stérile.
14° Ro.....	Fracture de l'humérus.....	Stérile.
15° Ca.....	Fracture du sternum.....	15 à 20 microbes par champ microscopique.
16° Gu.....	Fracture de cuisse. Traitement incomplet à cause de dysenterie bacillaire grave.....	40 à 100 microbes par champ microscopique.
17° Qu.....	Gangrène gazeuse.....	Fermeture par corsetage.
18° Ba.....	Infection gazeuse.....	Suturé.
19° Dr.....	Fracture des métacarpiens. Plaies des parties molles.....	Suturé.
20° Co.....	Fracture de cuisse.....	Presque stérile
21° Ro.....	Plaies des parties molles.....	Suturé.
22° As.....	Plaies des parties molles.....	Suturé.
23° Vu.....	Plaies des parties molles.....	Suturé.
24° Ma.....	Plaies des parties molles.....	Suturé.
25° Gu.....	Fracture du tibia.....	Stérile.
26° Sc.....	Plaies des parties molles.....	Suturé.

27° Sh.	Plaie pénétrante du genou.....	Suturé.
28° Bo.	Plaies des parties molles.....	Suturé.
29° Me.	Plaies des parties molles.....	Suturé.
30° Ma.	Fracture de l'humérus.....	Encore infecté. (20 à 30 par champ micro- scopique).
31° Th.	Fracture de l'omoplate.....	Suturé.
32° La.	Fracture de l'ischion, ouverture du rec- tum.....	Une plaie fermée, l'autre encore infectée (50 à 100).
33° Me.	Plaies des parties molles.....	Fermé.
34° Ro.	Plaies des parties molles.....	Stérile.

Sans nous attarder aux plaies des parties molles dont la stérilisation ne présente aucune difficulté réelle, nous allons donner ici, comme exemple des résultats obtenus, des observations empruntées à trois catégories de lésions particulièrement graves: les fractures, les plaies du genou, les gangrènes gazeuses.

1° *Fractures.*

L'évolution des fractures infectées, une fois la stérilisation acquise, diffère suivant que l'étendue des lésions des parties molles permet ou non la fermeture. Dans le premier cas on peut immédiatement après la suture appliquer un appareil définitif, faisant une contention exacte; en sorte, qu'à partir de ce moment, le traitement chirurgical proprement dit peut être considéré comme terminé. Au contraire, dans l'hypothèse d'une perte de substance des téguments rendant la suture impossible, il sera nécessaire de continuer l'irrigation, en la surveillant de très près, surtout pendant la période où le foyer de fracture communique encore avec l'extérieur.

(a) *Fractures qu'il est possible de fermer.*—Le temps nécessaire à la stérilisation d'un foyer de fracture infecté, varie suivant la nature de l'os atteint, et peut-être suivant la durée et la nature de l'infection. Les lésions du cubitus, du radius, du squelette du poignet et de la main, du péroné, du squelette du pied, de l'omoplate, etc... se stérilisent aisément, souvent en une dizaine de jours. Pour le tibia et le fémur il faut en général un temps plus long. L'humérus présente une difficulté intermédiaire. C'est ainsi que sur 5 fractures de la dia-

physe humérale, comprises dans de lot de 34 blessés qui sert de base à ce travail, 3 ont pu être fermées après 8 et 12 jours, tandis que les deux autres ont été stérilisées, l'une après 34 jours, l'autre incomplètement. Ces deux retards s'expliquent, dans un cas par une fracture articulaire, et dans l'autre cas, par une infection à streptocoque pur avec état général grave.

Pour faire saisir d'une manière directe la marche générale de la stérilisation et de la fermeture des fractures infectées, il nous paraît nécessaire de donner un type de chacune des catégories que nous avons eues à traiter.—Savoir:

Fracture de l'omoplate.....	1
— de l'humérus.....	1
— du radius.....	1
— du radius et du cubitus.....	1
— de cuisse.....	1
— de jambe.....	1

Observation I.—Fracture partielle de l'omoplate par éclat d'obus.

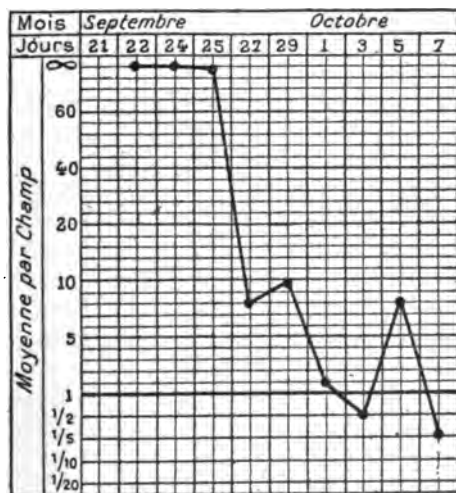


Fig. 1.—Courbe microbienne de l'obs. I¹.

1. Les chiffres qui représentent les moyennes par champ microscopique se lisent de bas en haut de la manière suivante: 1 bactérie pour 20 champs, 1 pour 10, 1 par champ, 5 par champ,, l'infini c'est-à-dire nombre de bactéries incomptable.

T. . . . Joseph, trente et un ans, 2^e colonial. Blessé le 17 septembre 1916 à 15 h. 30.—Opéré le 19 septembre après-midi dans une ambulance de la Somme.

Entré à l'hôpital 21, à Compiègne, le 21 septembre au soir, quatre jours après sa blessure.

La fiche porte: grande plaie scapulaire droite par éclat d'obus. A eu une hémorragie d'une artère scapulaire.—Liquide de Mencièr.

Examen à l'entrée.—Température 37°,7. Bon état général sauf la fatigue du transport. Teint très jaune.

Plaie importante de la région scapulaire et dorsale droite d'environ 25 cm. de long sur 10 de large, partant de la partie interne et supérieure de l'omoplate pour descendre en bas et en dedans vers la colonne vertébrale. Cette plaie intéresse, 1^o en haut, la partie interne de la base de l'épine de l'omoplate qui est fracturée et abrasée, 2^o au milieu, les muscles de la gouttière costo-vertébrale, 3^o en bas, une apophyse épineuse de la région dorsale. Odeur créosotée, aspect nécrotique sec et noirâtre surtout vers les bords. Lymphangite réticulaire de voisinage. Gonflement étendu.

Mise en irrigation discontinue à la liqueur Dakin. Détersion et désinfection d'abord lentes en raison de la présence de tissus sphacelés. A l'examen des frot-tis, nombre de microbes incomptable pendant les cinq premiers jours, tombant à 8 par champ microscopique le septième jour, à 2 le douzième, pour remonter à 8 le quatorzième (petit foyer microbien au niveau de la partie supérieure de la plaie mal irriguée). Le seizième jour de la désinfection, le nombre moyen des microbes des frottis étant à 1 par 5 champs de microscope examinés, on décide de fermer la plaie.

Fermeture au crin le seizième jour après l'entrée du blessé.—Ablation du liséré épidermique aux ciseaux courbes. Libération des bords. En décollant le bord interne dans une certaine étendue, on tombe sur du tissu sclérosé, lardacé par places, saignant au décollement, qui paraît répondre à l'organisation d'une infiltration hématiche et rend difficile la libération. On rapproche les bords à grand'-peine en raison de la perte de substance et du peu de souplesse des tissus. Sept jours après le rapprochement, tension de la cicatrice. Ablation des deux points inférieurs, il s'écoule une certaine quantité de sang. Pour plus de sûreté on introduit deux tubes d'irrigation qu'on enlève deux jours après. Ablation des derniers fils sans autre incident le onzième jour après la fermeture. La réunion reste complète. Le point de passage des deux tubes s'accole exactement.

24 octobre.—La suture tient parfaitement et le plan cutané adhère complètement au plan profond.

Observation II.—Fracture de l'humérus gauche à l'union du tiers supérieur et du tiers moyen, par éclat d'obus.

M. . . . Séraphin, trente-quatre ans, 21^e d'infanterie. Blessé le 24 août à 22 h. 30. Opéré le 25 dans une ambulance de la Somme.

Entré à l'hôpital 21, à Compiègne, le 5 septembre au soir, douze jours après sa blessure.

La fiche porte : séton du bras gauche; fracture de l'humérus gauche; plaie du mollet droit; par éclat d'obus.

Examen à l'entrée.—Température à 38°. Aspect fatigué.

1^o Bras gauche.—Au niveau du tiers moyen, plaie externe verticale longue d'environ 10 cm., large de 5 d'où sort une mèche iodoformée avec un gros drain. Immédiatement sous l'aisselle, plaie interne longue environ de 5 à 6 cm. Trajet très oblique réunissant les deux plaies en passant à travers un foyer de fracture à l'union du tiers supérieur et du tiers moyen. Les plaies sont sanieuses, odorantes, particulièrement l'externe, et les compresses qui les recouvrent sont imbibées de pus bleu. Chute du poignet. Paralyse du nerf radial.

A l'examen radiographique, fracture oblique avec un fragment supérieur d'autant plus oblique qu'une esquille postérieure assez importante s'en détache en dehors.

2^o Jambe droite.—Plaie en séton du mollet passant entre les muscles postérieurs de la jambe et le squelette. Mollet souple.

Mise en irrigation discontinue à la liqueur de Dakin.

Deux jours après, disparition du pus bleu au niveau du bras. Les sécrétions diminuent. Les plaies ont pris l'aspect rouge habituel. A la jambe droite un peu de pus contenant des débris de vêtements. Ni douleur ni gonflement. La plaie externe du bras, qui, le premier jour, montrait sur des frottis un nombre incommensurable de microbes, n'en avait plus que 3 par champ microscopique au bout de deux jours, et, le dixième jour après l'entrée, le nombre des microbes tombait, à 1 par 5 champs. On attendit encore deux jours et on fit 2 nouveaux examens avant de fermer. De son côté la plaie de l'aisselle présentait un microbe par 2 champs le premier jour et seulement 1 par 20 champs le sixième. Au mollet, à l'entrée, 60 microbes par champ dans une plaie, 40 dans l'autre. Désinfection acquise le sixième jour. Fermeture complète aux crins des plaies du bras et du mollet le 17 septembre, douze jours après l'entrée du blessé. Mise en plâtre (Hennequin modifié) du bras gauche deux jours après. Ablation des fils le dixième jour. Réunion complète par première intention des plaies du bras et du mollet, maintenue depuis lors.

Le 24 octobre, ablation du plâtre. Découverte et libération du nerf radial qui est rejeté en avant et inclus dans la partie superficielle du col. Il y a continuité du nerf, mais un petit névrome allongé se trouve à la partie supérieure de la portion intéressée. Réfection d'un plan musculaire au-dessous et au-dessus du nerf. Suture aux crins.

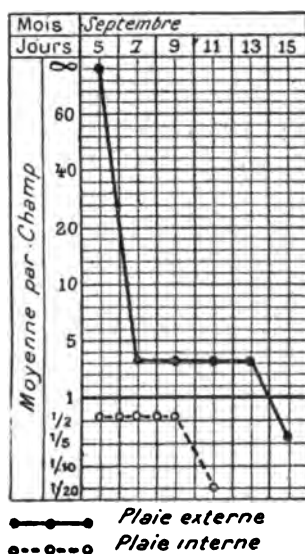


Fig. 2.—Courbe microbienne de l'obs. II.

Il est intéressant de noter qu'une opération nécessitant une rigoureuse asepsie a pu être faite au niveau même d'une des plaies récemment refermées.

Observation III.—Fracture du radius droit avec ouverture de l'articulation du coude par éclat d'obus.

J... Louis, vingt ans, 109^e d'infanterie. Blessé le 23 août à dix-sept heures. Opéré le 23 août à dix-neuf heures dans une ambulance chirurgicale automobile.

Entré à l'hôpital 21 à Compiègne, le 5 septembre, treize jours après sa blessure.

La fiche porte: Plaies multiples par éclats d'obus. 1^o Séton du coude droit. Fracture du col radial avec ouverture de l'articulation. Grosse lésion des muscles

antérieurs du pli du coude. Les vaisseaux sont mis à nu, mais ne paraissent pas blessés. Résection de la tête radiale. Mèche d'éther. 2^o Séton de la cuisse gauche. Large débridement. 3^o Séton de la main droite avec fractures métacarpiennes.

Examen à l'entrée. Température 37°,8. Bon état général.

Avant-bras droit. A la partie supérieure et externe, légèrement en arrière, plaie d'environ 10 cm. de long. En avant et en dedans plaie d'environ 5 cm. de long. Ces deux plaies sont en pleine suppuration mais ont bon aspect. A la radiographie la tête et le col du radius ont complètement disparu.

Séton de la main droite. Suppuration abondante.

Large plaie de la partie supérieure de la cuisse gauche.

Mise en irrigation discontinue à la liqueur de Dakin.

Les plaies cessent progressivement de suppurer. Le nombre des microbes passe, dans la

plaie externe de l'avant-bras de 35 au premier jour à 1 par 4 champs au dixième, dans la plaie interne de 8 par champ à 1 par 20 champs le quatrième jour.

Fermeture complète au crin des plaies de l'avant-bras et de la cuisse le 17 septembre, douze jours après l'arrivée du blessé.

Ablation des fils, dix jours après. Réunion *per primam*. Coude mobile.

Observation IV.—Fracture des deux os de l'avant-bras avec vaste perte de substance par éclat d'obus.

G... Auguste, trente-neuf ans, 2^e colonial. Blessé le 27 août à neuf heures.

Opéré le même jour à dix-neuf heures dans une ambulance de la Somme.

Entré à l'hôpital 21, à Compiègne, le 5 septembre au soir.

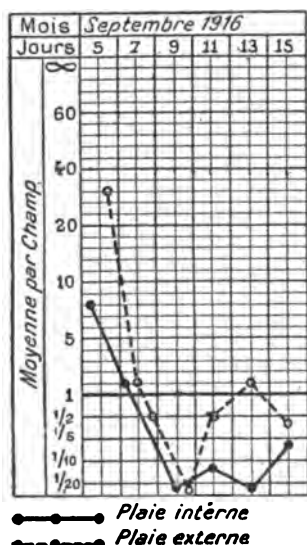


Fig. 3.—Courbe microbienne de l'obs. III.

La fiche porte: Vaste plaie de la face postérieure de l'avant-bras gauche à quelques centimètres au-dessous du coude avec section complète des muscles de la face postérieure et broiement des deux os. Nettoyage des foyers de fractures. Ablation des esquilles, régularisation des fragments. Nettoyage des parties molles. Pas de lésions vasculaires. Pansement au liquide de Mencièr. Immobilisation.

Examen à l'entrée. Température 38°,3. Aspect fatigué. Teint jaune. Large plaie de la face postérieure de l'avant-bras gauche tiers supérieur, longue d'environ 12 cm., large d'autant, intéressant les trois cinquièmes de la circonférence de l'avant-bras.

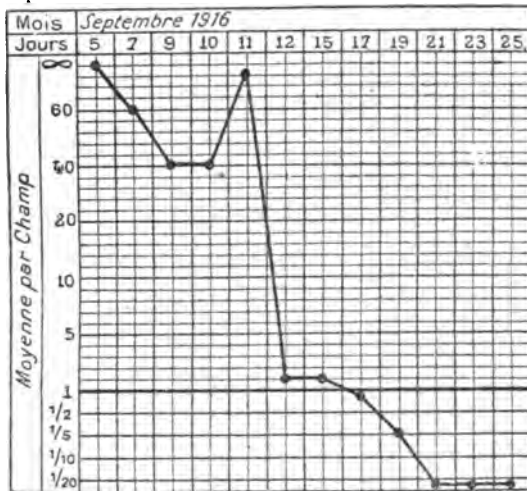


Fig. 4.—Courbe microbienne de l'obs. IV.

Fracture complète des deux os de l'avant-bras avec perte de substance de 2 cm. environ sur chacun d'eux. Aspect nécrotique de la surface et des bords de la plaie. Fragments de tissus sphacelés. Compresses imbibées de pyocyanique. Main et avant-bras oedématisés. Sur le pourtour de la partie inférieure du bras irritation étendue de la peau avec petites ulcérations partielles. Le pansement enlevé datait de la veille et était le troisième depuis l'opération.

Le deuxième pansement était resté en place sept jours et à son ouverture, on aurait constaté, au dire du blessé, la présence de vers.

Mise en désinfection chlorée. Le lendenain pus bleu encore abondant.

Le 7, deux jours après l'arrivée, disparition du pyocyanique. La plaie est encore couverte de pus. D'innombrables à l'entrée, les microbes deviennent numérables. On en évalue à peu près 60 par champ. L'oedème de l'avant-bras diminue.

Le 9, les débris de tissu sphacelés s'éliminent. La température remonte de 38° à 38°,5.

Le douzième jour, l'aspect est celui d'une plaie détergée et rouge.

Le 17, la surface de la plaie est couverte de granulations rouges et lisses. On ne trouve plus de microbes que sur les surfaces osseuses: un par champ en moyenne.

Le 19, 1 microbe par 5 champs.

En raison de l'infection de la plaie à l'arrivée, au niveau des parties molles et au niveau des surfaces osseuses, on fait encore des prises régulières jusqu'au 25.

Le 25 septembre, vingt jours après l'entrée, on ferme complètement au crin. La perte de substance étant très étendue, il faut largement libérer les bords par en haut et par en bas. Les tissus tirent fortement.

Ablation des fils dix jours après. Plusieurs ont coupé en raison de la traction des tissus. En un point de la suture, sur un demi-centimètre, petite escarre qui tombe.

Observation V.—Fracture trans-trochantérienne de la cuisse gauche par balle.

L... Gaston, trente et un ans, 17^e d'infanterie. Blessé le 24 août à six heures du matin. Opéré le même jour après midi dans une ambulance de la Somme. Forte hémorragie avant et pendant l'opération.

Entré à l'hôpital, 21, à Compiègne, le 5 septembre au soir, douze jours après sa blessure.

La fiche porte: Blessure par balle. Plaie en sétou. Orifice d'entrée à la marge de l'anus. Orifice de sortie à la face antéro-externe de la cuisse en avant du trochanter. Fracture des os du bassin et de la cuisse gauche. Huile camphrée, éther.

Examen à l'entrée.—Température 37°,8. Etat général assez satisfaisant. Le blessé est dans un grand plâtre qu'on enlève.

La plaie de la marge de l'anus est fermée. A la face antéro-externe de la cuisse gauche plaie d'environ 10 centimètres de long sur 4 de large, à bords épidermisés, située en avant et en bas de la région trochantérienne. Cette plaie conduit dans un trajet profond et irrégulier, donnant un liquide purulent, mais d'abondance modérée. Gonflement et douleur du pli de l'aîne, mais sans réaction inflammatoire intense.

On se contente d'immobiliser le blessé avec un appareil de Tillaux et d'introduire profondément dans les différents points du trajet trois tubes d'irrigation.

Mise en irrigation discontinue à la liqueur de Dakin.

Radiographie quatre jours après l'entrée.

Les os du bassin paraissent intacts. Au niveau de la partie supérieure de fémur droit, immédiatement au-dessous du grand trochanter, trait de fracture horizontal et irrégulier intéressant en dedans le petit trochanter. Engrènement des fragments et nombreuses petites esquilles. De la partie externe particulièrement irrégulière du trait de fracture monte, en haut et en dehors, un trait oblique qui détache la base et la partie externe du trochanter. Disséminés dans les tissus voisins, cinq éclats métalliques du volume apparent d'un gros pois.

L'examen bactériologique fait en différents points du trajet montre trente microbes par champ. Le 1^{er} octobre, le taux est de un microbe par champs.

Ce jour même, 25 jours après l'entrée, on ferme complètement aux crins de Florence. Ablation des fils dix hours après. Réunion *per priman*.

Observation VI.—Fracture de la jambe gauche à son extrémité inférieure par balle.

D... Yves, trente-deux ans, 268° d'infanterie. Blessé le 31 août à dix-huit heures trente. Opéré le 1^{er} septembre dans une ambulance de la Somme.

Entré à l'hôpital 21, à Compiègne, le 5 septembre à six heures du soir, cinq jours après sa blessure.

La fiche porte: Plaie en séton de la jambe gauche par balle de mitrailleuse. Orifice d'entrée à la base de la malléole interne. Orifice de sortie à la face antérieure du cou-de-pied. Il y a une fracture sus-malléolaire du tibia. Intervention: Débridement du trajet. Nettoyage de celui-ci et du foyer de fracture à l'éther. Mèches. Pansement.

Examen à l'entrée. Température 37°, 2. État général satisfaisant.

1° A la partie supérieure de la jambe gauche, intéressant également la région du cou-de-pied et la région malléolaire interne, vaste plaie des parties molles avec au fond une cavité osseuse anfractueuse, répondant à la base de la malléole interne face antérieure. Les compresses qui bourrent cette cavité sont odorantes mais peu souillées car toute la plaie a bon aspect. Les tissus environnants sont souples.

2° Plaie d'environ 3 centimètres de long à la base de la malléole externe en dehors et en arrière, d'aspect très net.

A la radiographie: fracture très oblique du tibia partant de la base de la malléole et montant à 10 centimètres sur le diaphyse. Trait de fracture ouvrant l'articulation tibio-tarsienne. Fracture du péroné à la base de la malléole externe. Le pied est fortement déplacé en dedans et en avant.

Mise en désinfection chlorée. Nombre peu considérable de microbes. Dès le 11 septembre la plaie est stérilisée.

Fermeture aux crins le 11, six jours après l'entrée du blessé. En raison de la large perte cutanée, mobilisation des deux lèvres de la plaie en décollant le tissu cellulaire jusqu'à la face postérieure de la jambe. L'ensemble des sutures tire un peu.

Ablation des fils le dixième jour. A la partie supérieure, un des bords de la plaie s'est légèrement sphacélé sur une petite étendue.

(b) *Fractures qu'il n'est pas possible de fermer.*—Nous avons dit que dans certains cas les lésions des parties molles ont une étendue qui rend impossible toute réunion, alors que la stérilisation est acquise. En voici un exemple:

Observation VII.—Fracture comminutive du tibia droit au tiers supérieur par éclat de torpille.

F... Louis, trente-huit ans, 264° d'infanterie, blessé le 1^{er} septembre à dix-huit heures. Opéré le 2 septembre à deux heures dans une ambulance chirurgicale automobile. Entré à l'hôpital 21, à Compiègne, le 5 septembre au soir.

La fiche porte: fracture de la jambe droite au tiers supérieur; vaste broiement du tibia dont on enlève de nombreuses esquilles libres. Plusieurs adhérentes au périoste de la face interne, quoique mobiles sont laissées soigneusement en place. Désinfection et pansement à l'éther. Immobilisation dans une gouttière métallique.

Examen à l'entrée.—Température 38°,6. Aspect très fatigué, teint jaunâtre. Très large plaie de la partie supérieure et antérieure de la jambe droite d'environ 15 centimètres de long sur 10 de large en haut; perte de substance du tibia de 6 centimètres de long; les muscles ont bonne apparence, mais la surface du tibia est inoculée et noirâtre, particulièrement au niveau du fragment supérieur dont une



Fig. 5.—Courbe microbienne de l'obs. VII.

partie forme surface irrégulière de section et dont une autre plus superficielle est dénudée. Gonflement marqué des tissus environnants.

La radiographie confirme la perte de substance tibiale qui existe en réalité sur plus de 8 centimètres; un biseau irrégulier du fragment supérieur et du fragment inférieur maintenant en partie la continuité du côté interne. Une grosse et courte esquille oblique est enfoncée dans les tissus.

Mise en irrigation discontinue à la liqueur de Dakin. La température persiste pendant trois jours, oscillant entre 38 et 39°, et ne se rapproche de la normale que vers le 10. A ce moment l'œdème de la jambe a disparu. La partie dénudée du fragment supérieur a un aspect rosé, mais les surfaces de section osseuses sont encore nécrosées et noirâtres. Courbe microbienne: microbes incomptables.

Puis l'aspect de la plaie s'améliore, mais la courbe microbienne ne descend à 5 microbes par champ que pour remonter à plus de 60.

Malgré une nouvelle diminution, d'autres oscillations du nombre des bactéries restant à craindre, on décide le 27 d'enlever l'esquille interne nécrosée et les surfaces lamellaires noirâtres sous lesquelles se conservent les foyers d'infection. L'opération est faite en ménageant le périoste avec le plus grand soin.

L'intervention n'est pas suivie de réaction inflammatoire avec température, mais simplement d'une poussée microbienne constatable par les frottis et qui dure sept jours. Cependant les surfaces osseuses se recouvrent de bourgeons, la courbe microbienne descend rapidement, et après plusieurs oscillations atteint le 19 octobre 1 microbe par 5 champs.

A ce moment quarante-trois jours après l'entrée du blessé, la plaie est pratiquement stérile et pourrait être refermée. L'étendue de la perte de substance des parties molles et le peu de mobilité que présentent les tissus au niveau de l'extrémité supérieure de la jambe, surtout lorsqu'ils ont subi une poussée inflammatoire l'interdisent complètement. Il faut se résigner à laisser la plaie se combler spontanément, puis se cicatriser en surface, tout en la maintenant stérile.

2^o Plaies du genou.

Observation VIII.—Plaie pénétrante du genou gauche par éclat d'obus.

S... Jacques, vingt-huit ans, 352^e régiment. Blessé le 13 septembre à quatorze heures. Entré à l'hôpital 21 à Compiègne, le 15 septembre à quatorze heures trente, quarante-huit heures après sa blessure.

Examen à l'entrée.—Température 38°,3. Le blessé se plaint assez fortement. Plaie peu étendue de la région antéro-interne du genou gauche à la base de la rotule. Genou globuleux et douloureux, mais sans empâtement lymphangitique.

A la radiographie, éclat d'obus de la grosseur d'un haricot localisé dans le plateau tibial interne.

Opération le 15 septembre à seize heures.—Anesthésie éther. Incision verticale passant par l'orifice d'entrée. Excision des tissus contus. On constate que la synoviale est ouverte. Agrandissement de l'orifice. Il s'en écoule environ 100 g. de liquide sanguinolent trouble. Extraction du projectile. Ablation à la pince-gouge des tissus osseux lésés. Cloisonnement de la synoviale avec deux compresses.

Mise en irrigation discontinue à la liqueur de Dakin de la partie interne de l'articulation, en même temps qu'on installe une traction continue de 5 kilos.

La désinfection de la plaie est obtenue au bout de douze jours. Au sixième jour on a enlevé les compresses de cloisonnement.

Fermeture complète aux crins le 28 septembre, treize jours après l'arrivée du blessé. Ablation des fils le 8 octobre. Le 23, le blessé fléchit le genou à 45°.

3^o Gangrènes gazeuses.

Sur nos 34 blessés nous avons observé deux fois cette redoutable complication. Dans un cas, l'infection a été saisie à son début,

alors qu'elle était encore localisée à la loge antéro-externe de la jambe. Dans l'autre, il s'agissait de lésions diffuses tellement étendues que la stérilisation et les manœuvres nécessaires à préparer une autoplastie retardèrent notablement la fermeture.

Observation IX.—Foyer gangréneux avec gaz de la jambe droite.

B... Victor, vingt et un ans, 31^e bataillon de chasseurs. Blessé le 4 septembre à quatorze heures. Opéré partiellement, avant son entrée, dans une ambulance de la Somme.

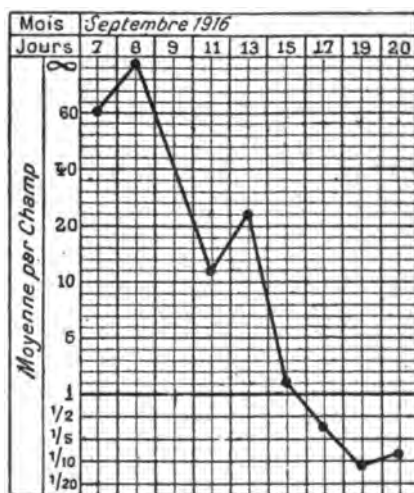


Fig. 6.—Courbe microbienne de l'obs. IX.

Entré à l'hôpital 21, à Compiègne, le 6 septembre au soir, deux jours après sa blessure.

La fiche porte: Plaies multiples des deux bras, de la face, du pied droit, de la jambe droite et du mollet gauche par éclats d'obus. *Radiographie:* Les éclats sont enlevés. Nettoyage.

Examen à l'entrée.—Température 38°9. Aspect très fatigué. Le blessé est obnubilé, somnolent, ne répondant pas. On se contente de renouveler rapidement le pansement, en cherchant à glisser des tubes d'irrigation dans les orifices des plaies les plus importantes.

Les jambes, les bras, la face sont en effet criblés d'orifices dont un certain nombre ont été plus ou moins complètement débridés.

A la jambe droite en particulier trajet vertical en sêton de la partie moyenne et antérieure de la jambe; membre tendu et gonflé.

Le lendemain matin, 7 septembre, le gonflement est encore plus accentué, il s'étend jusqu'au dos du pied et remonte jusqu'à l'extrémité supérieure de la jambe. Le trajet crépite dès qu'on le palpe et des gas sortent avec de la sanie par les orifices.

Opération le 7 septembre.—Anesthésie à l'éther. Débridement d'un orifice à l'autre. Le jambier antérieur est entièrement sphacélé de son extrémité supérieure jusqu'à son insertion tendineuse.

Ablation du jambier et nettoyage de toute la loge dont la paroi reste garnie de tissus sphacelés et odorants.

Les frottements faits avec l'exsudat montrent des bacilles abondants sans caractère particulier. Le culture donne un bacille du type *perfringens* associé à d'autres bactéries.

Mis en irrigation discontinue à la liqueur de Dakin.

Dès le 9, l'œdème du dos du pied a disparu, et les bords du foyer gangréneux se détachent.

Le 11 le tissu rosé apparaît partout, sauf dans un petit espace du fond. Les microbes d'abord incompressibles tombent à 12 par champ.

La désinfection et la détersion se poursuivent régulièrement. Le 17 on compte 1 microbe par 3 champs. En raison de l'état de septicité antérieure de la plaie, des prises microbiennes sont encore faites régulièrement pendant cinq jours. Le taux des microbes descend à 1 par 10 champs. Fermeture complète au crin le 22 septembre, seize jours après l'entrée. Réunion *per primam*.

Observation X.—Gangrène gazeuse de la fesse gauche à forme extensive.

Q. - - - Jean, trente-six ans, 219^e d'infanterie. Blessé le 4 septembre à dix-sept heures.

Entré à l'hôpital 21, à Compiègne, le 6 septembre au soir, deux jours après sa blessure. C'est à cause de son état général grave et de l'odeur infecte de ses plaies qu'en cours de route on l'a fait descendre du train qui l'évacuait sur Paris.

La fiche porte: Plaie par éclat d'obus de la fesse gauche. Lésion probable du gros intestin.

Examen à l'entrée.—Température 39°, 2. Aspect grave. Subictère. Obnubilation. Pouls petit. Langue sèche.

Orifice d'entrée à la partie inférieure de la fesse gauche. Orifice de sortie contre la crête iliaque. La fesse est distendue et couverte de phlyctènes. Le bourrelet qui limite cette distension s'étend en haut jusqu'au flanc, et en bas sur la cuisse.

Crépitation gazeuse étendue. Odeur caractéristique des plus prononcée.

Opération immédiate le 6 septembre à huit heures du soir.

Anesthésie à l'éther. Incision passant par les deux orifices. Dissection d'un grand lambeau antérieur comprenant la presque totalité du grand fessier que l'on libère et qu'on enlève. Toute la fesse a l'aspect sphacélé et délignescent. Nettoyage d'une fusée entre le moyen et le petit fessier. On tente d'enlever l'enduit diphtéroïde qui recouvre les surfaces exposées mais on est arrêté par une hémorragie abondante. On fait à la peau du lambeau quatre incisions peut-être inu-

tiles, car il est facile, comme on s'en aperçoit secondairement de nettoyer le derme par sa profondeur. Il n'y a pas de trajet communiquant avec l'abdomen, à plus forte raison avec l'intestin.

Mise en irrigation discontinuée à la liqueur de Dakin. On dispose treize tubes d'irrigation sur la surface de la plaie qui, le lambeau n'étant pas remis en place, présente près de 50 cm. de long sur 40 de large.

Les frottis montrent des bacilles sporulés avec des spores libres. La culture donne un bacille du type *perfringens* associé à d'autres bactéries.

Le 8, la section postérieure du grand fessier commence à se déterger, le moyen fessier également.

La température baisse un peu ($38^{\circ},5$), le blessé est moins absorbé, la langue est humide. La détersion se poursuit et le 11 septembre, la plaie sauf en des

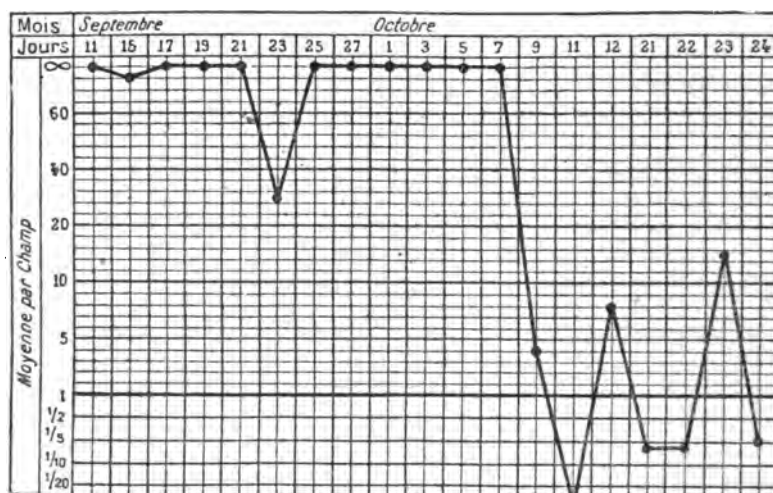


Fig. 7.—Courbe microbienne de l'obs. X.

points très localisés, a un aspect absolument frais et rouge. Puis la température qui le 11 était revenue à la normale se met à osciller entre 38° et $38^{\circ},5$, remontant au voisinage de 40° . Le 24 septembre on enlève sous le lambeau supérieur des tissus sphacelés en même temps qu'on découvre deux collections purulentes, l'une vers la masse sacrolombaire, l'autre vers la partie externe de la cuisse. La température baisse alors progressivement.

Malgré l'aspect frais et détergé des différentes parties de la plaie, ce n'est seulement que le 7 octobre que la courbe microbienne quitte l'infini. Elle descend brusquement alors à 1 microbe par 20 champs. La raison de cette chute est que les prises étaient faites sur les points sphacelés et qu'à ce moment même ces points disparaissent complètement.

Le 11 octobre on commence à rapprocher les deux lèvres de la plaie avec des bandelettes d'adhésif. Les tissus, d'abord sans souplesse, se prêtent peu à peu et les bords se rapprochent sur toute la longueur, sauf à la partie supérieure. Pendant ce rapprochement progressif on cesse l'irrigation, que l'on reprend à partir du 20 octobre en raison d'une légère réinfection de la plaie due à l'application des bandelettes.

Le 24 octobre, traction élastique sur les bords de la plaie par le procédé du "corsetage."

Technique.

On peut dire que le seul problème qui se pose est de mettre en contact avec l'antiseptique toutes les parties de la plaie. Pour cela il est nécessaire d'avoir des débridements aussi complets que possible. C'est ce que nous réalisons sur les blessés frais (blessés de 24 heures).

La première idée qui se présente en face des blessures infectées datant de plusieurs jours est donc d'opérer dès son entrée tout malade dont les plaies, et particulièrement les foyers de fracture, ont été insuffisamment ouverts.

Cette manière de faire est un devoir dans les cas où l'abstention entraînerait la mort rapide. Il en va ainsi pour les hématomes liés à la lésion d'un gros vaisseau, les infections gangréneuses avec gaz, les foyers de fracture infectés s'accompagnant d'état général grave et n'ayant au niveau des téguments que des orifices insuffisants pour l'irrigation. Dans les autres cas il convient de s'abstenir de toute intervention précoce.

Les opérations complètes pratiquées sur des plaies infectées, surtout quand il s'agit de fractures, présentent, en effet, une très grande gravité. Tout récemment, un chirurgien rapportait une série de cinq *esquillectomies secondaires précoces* faites sur des blessés atteints de fracture de cuisse déjà opérés. Tous moururent.

Le contrôle bactériologique qui est exercé sur chacune des plaies que nous traitons, permet d'affirmer que toute intervention faite dans un foyer de fracture, même en voie de stérilisation, est suivie, d'une manière constante, d'un relèvement formidable du nombre des bactéries. Pour fixer les idées, la courbe bactériologique d'un foyer de fracture qui donne en moyenne 10 bactéries par champ microscopique, passera, après opération, et en quelques heures, à l'infini. On peut

juger par là de la poussée microbienne qui suit une intervention considérée, faite en milieu très septique sur un malade affaibli.

La courbe bactériologique qui accompagne l'observation VII en fournit un excellent exemple. Le 27 septembre le nombre des bactéries est tombé à 6 par champ. Le même jour on intervient pour enlever des segments osseux nécrosés qui retardent la stérilisation. Immédiatement le nombre des bactéries passe à l'infini.

Il faut donc toutes les fois qu'il y a vers la profondeur des plaies un accès permettant une irrigation, même incomplète, tenter une première désinfection. Dans la règle, en agissant ainsi, on réussit à mettre les blessés dans une condition telle, que des opérations complémentaires peuvent être pratiquées, sans risques. Le fait que le plus grand nombre des plaies évacuées du front vers l'arrière, sont débridées, rend très aisée l'application de ce principe.

Dans ces conditions le schéma du traitement d'un blessé infecté depuis plusieurs jours, exception faite pour les cas d'urgence réservés plus haut, devient le suivant:

1° Désinfection primitive; 2° Intervention s'il y a lieu; 3° Stérilisation; 4° Fermeture.

Le passage d'un de ces stades au suivant n'est pas livré au hasard. Il se fait d'une manière automatique en suivant la marche bactériologique de chaque plaie.

C'est ici le lieu de rappeler le point capital de la méthode de Carrel. Il a été publié maintes fois, mais souvent insuffisamment compris.

Tous les deux jours au moins, en prenant la précaution d'arrêter l'irrigation deux heures plus tôt, on fait un frottis avec l'exsudat de chacune des plaies en traitement. La prise doit être faite dans les points les plus suspects, qui sont les plaques de sphacèle et les os dénudés. Sur chaque frottis on compte les bactéries d'au moins trois champs microscopiques. La moyenne donne un chiffre que l'on porte sur une courbe. Il est important de représenter par une courbe l'évolution bactériologique d'une plaie, car on saisit toujours mal, sur une série de chiffres, la marche d'un phénomène. Quand la plaie tombe à un petit nombre de bactéries par champ, on doit faire les prises chaque jour, en les multipliant. L'expérience prouve que la

fermeture des plaies peut être réalisée, dès que l'on ne trouve plus qu'une bactérie par trois ou quatre champs.

Il y a dans la méthode de Carrel un élément qui égale en importance le contrôle bactériologique: c'est la préparation correcte du liquide de Dakin. Bien qu'on ait souvent insisté sur ce point, nous ne craignons pas d'y revenir.

La solution de Dakin possède deux caractéristiques qui sont: 1° une teneur en hypochlorite de soude comprise entre 0,45 et 0,50 p. 100; 2° l'absence de soude caustique.

Pour satisfaire à la première condition, il est indispensable de partir d'un chlorure de chaux dont le titre en chlore actif soit exactement connu. De cette teneur en chlore actif dépendra la quantité des différents produits à employer pour la préparation.

La seconde condition est réalisée dans le mode de préparation qui a été préconisé par Daufresne, grâce à la substitution d'une certaine quantité de bicarbonate de soude à une partie du carbonate de soude nécessaire à la réaction.¹

Toute tentative de stérilisation des plaies faite avec un liquide ne répondant pas à ces conditions conduit à un échec certain.

Ceci posé, voyons comment les choses se passent dans la pratique, en prenant comme type une fracture déjà opérée depuis plusieurs jours et arrivée infectée.

Par ses incisions conduisant dans le foyer, l'irrigation est établie. Au bout d'un temps variable, souvent très court, le pus disparaît et le nombre des microbes tombe. Si alors la courbe descend d'une façon régulière, l'irrigation est continuée sans modification. Si, au contraire, elle se relève pendant plusieurs jours en formant un plateau, c'est qu'il y a dans la plaie une cause d'infection: débris de vêtements, tissus sphacelés, ostéite, séquestre, et qu'il faut intervenir pour la supprimer.

La désinfection primitive a donné ce qu'elle pouvait; attendre davantage serait, non seulement perdre du temps, ce qui est grave, mais risquer l'augmentation du sphacèle ou de l'ostéite.

1. M. Daufresne, Mode de préparation de l'hypochlorite de soude chirurgical; différence entre la solution de Dakin et celle de Labarraque, *Presse Médicale*, 23 octobre 1916.

L'opération faite, l'irrigation est reprise jusqu'au degré de stérilisation qui permet la fermeture.¹

Il va sans dire que, pendant ce traitement, les moyens ordinaires d'extension et de contention des fractures sont employés. Tout le temps que dure la période d'irrigation, nous renonçons complètement aux plâtres et nous employons les appareils américains qui permettent à la fois l'extension et la suspension dans un hamac. Après la suture, le traitement est établi comme pour une fracture fermée.

Nous possédons donc une méthode, qui, loin de nous laisser passifs en face d'une plaie suppurante, nous permet de ne pas attendre du hasard une fermeture spontanée, toujours douteuse. Grâce à la courbe bactériologique qui nous renseigne sur la marche de la désinfection et nous indique le moment propice à l'acte opératoire, nous sommes en mesure d'aborder d'une façon réglée le traitement des blessures de guerre infectées. C'est la possibilité de supprimer la suppuration dans les hôpitaux de l'arrière et d'aboutir rapidement à la fermeture chirurgicale des plaies, seul moyen de les mettre à l'abri de toute réinoculation.

1. Une bactérie pour trois champs microscopiques.

THE RÔLE OF YEAST IN THE NUTRITION OF AN INSECT (DROSOPHILA).

By J. H. NORTHROP.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

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It has been found by several authors¹ that the fruit fly, *Drosophila ampelophila*, when freed from microorganisms and raised under aseptic conditions grows much more normally on fruit or potato when yeast is present. This applies strictly, however, only to the growth of the larvæ, it having been found² that the imago is able to live the normal length of time on food which contains no yeast and which is inadequate for the growth of the larvæ. This is in accord with the results of McCollum³ and Osborne and Mendel,⁴ who have found that food which is insufficient for the growth of rats may be sufficient for their maintenance. It is well known from the work of Funk,⁵ Hopkins,⁶ Osborne and Mendel,⁷ McCollum,⁸ and others, that certain accessory food substances are required for the growth of rats, pigeons, chickens, and probably the higher animals in general. It has further been shown that yeast is especially rich in these substances. It seemed of importance, therefore, to determine whether in the case of *Drosophila* the yeast was needed to supply some ac-

¹ Guyénot, E., *Compt. rend. Soc. biol.*, 1913, lxxiv, 178, 223. Loeb, J., and Northrop, J. H., *J. Biol. Chem.*, 1916, xxvii, 309.

² Loeb and Northrop, *Proc. Nat. Acad. Sc.*, 1917, iii, 382.

³ McCollum, E. V., *Am. J. Physiol.*, 1911-12, xxix, 215.

⁴ Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1915, xxiii, 439.

⁵ Funk, C., *Die Vitamine*, Wiesbaden, 1914. See also *J. Physiol.*, 1912-17, and *J. Biol. Chem.*, 1913-17.

⁶ Hopkins, F. G., *J. Physiol.*, 1912, xlv, 425.

⁷ Osborne and Mendel, *Carnegie Institution of Washington, Publication 156*, 1911, pts. i and ii, and *J. Biol. Chem.*, 1912-17.

⁸ McCollum and collaborators, *J. Biol. Chem.*, 1913-17.

cessory food substance or whether the insects lived exclusively on yeast. Since it is impossible to conduct direct metabolism experiments on a single insect it was necessary to use indirect methods. This has been done in two ways; (1) by determining the number of flies that may be raised on a definite mixture of yeast and banana or yeast and casein, and (2) by determining the rate of growth of the larvæ on such mixtures.

1. In the first series of experiments 0.025 gm. of banana was weighed into each of fifteen test-tubes. Similar series were prepared containing 0.025 gm. of (a) casein, (b) sugar, and (c) 0.025 gm. of each. 0.25 cc. of a suspension of yeast in water containing 10 gm. of yeast⁹ in 100 cc. was added to each tube, the contents were thoroughly mixed, and sufficient absorbent cotton was added to absorb the excess water and prevent the flies from drowning. The tubes were then plugged with cotton and sterilized in the autoclave for 20 minutes. A similar series was prepared in which 0.25 cc. of water was added to the banana, etc., and also one containing the yeast suspension alone. Five to ten pairs of flies were then put in each tube (using the technique of Delcourt and Guyénot¹⁰) and allowed to remain until 50 to 100 eggs had been laid. This required 2 to 3 days. The flies were then removed and the tubes incubated at 26°. The number of flies which developed were counted. At the end of the experiment the tubes were tested for sterility by inoculation into glucose broth, agar, litmus milk, and yeast extract culture media. Only those tubes which remained sterile were considered in recording the results.

The numbers in Table I refer to the maximum number of flies which developed on any one culture of the series.

The results show that the number of flies which are able to develop on a certain quantity of yeast may be greatly increased by the addition of casein, sugar, or banana to the yeast. These substances must therefore be able to serve as food for the larvæ when yeast is also present. The fact that they are of little or no value when not mixed with yeast shows that the latter supplies some substance or substances which are necessary for the growth of the larvæ and which it cannot obtain, in sufficient quantity at least, from banana, casein, or sugar.

The quantitative results may be seen more clearly from the second series of experiments (Table II) in which the time required for the

⁹ The yeast used in all these experiments was free from starch and was obtained through the courtesy of the Fleischmann Company.

¹⁰ Delcourt, A., and Guyénot, E., *Bull. Sc. France et Belgique*, 1911, xlv, 249.

TABLE I.

Yeast.	Banana.	Casein.	Sugar.	No. of flies.
gm.	gm.	gm.	gm.	
O - O25				4
O - O25	0.025			9
	0.025			0*
O - O25			0.025	7
O - O25		0.025		16
O - O25		0.025	0.025	24
		0.025	0.025	0**

* If a large number of eggs are allowed to be laid on a considerable quantity of banana a few small sexually sterile flies¹¹ may be raised. If the banana is removed aseptically from the fresh fruit and kept sterile without heating, slightly better results are obtained, although it has thus far been impossible to raise a second generation even by this method.

** The ash from 0.025 gm. of yeast was added to this culture.

larvæ to reach the pupal stage is used as the index of the food value of the mixture.

2. In these experiments the cultures were made in 120 cc. Erlenmeyer flasks plugged with cotton and containing 8 gm. of the mixture. The yeast was added in the form of a 10 per cent suspension and thoroughly mixed with the banana. The flies were only allowed to remain in the flasks for 14 hours and were then removed and the cultures kept at 30°.

TABLE II.

Ratio, banana to yeast.	Time from egg to pupæ.	Time as pupæ.
	days	days
0	4.12 ± 0.04	3.43 ± 0.05
1	3.77 ± 0.02	3.62 ± 0.07
2	3.60 ± 0.01	3.68 ± 0.04
4	3.74 ± 0.02	3.54 ± 0.05
8	4.33 ± 0.03	3.49 ± 0.05
16	4.75 ± 0.03	3.23 ± 0.10
32	5.33 ± 0.08	3.45 ± 0.15
64	6.68 ± 0.16	Irregular; flies.
128	8.3 ± 0.2	Abnormal.
256	9.0 ± 0.2	
∞	14-25	

¹¹ Loeb and Northrop, *J. Biol. Chem.*, 1916, xxvii, 309. See also Guyénot,¹ 271.

The figures given in Table II are the average times required for all the larvæ to reach the pupal or imago stage (generally 100 to 200). It will be seen that the number of days required for the pupal stage to be reached decreases slightly up to a value for the ratio of banana to yeast of about two, and then increases rather rapidly. This seems to indicate that such a mixture allows a slightly more rapid growth of the larvæ than does yeast alone. As the proportion of banana to yeast becomes larger the results become more irregular, as is shown by the increase in the probable error, until when banana alone is used the average is of little significance. This increase is obviously due to the dilution of the yeast by the banana which, when present in excess, behaves as unavailable material. The irregularity in the results with large proportions of banana is partially due to the fact that as the value of the ratio of banana to yeast increases development becomes abnormal.

The pupal period remains the same, within the limits of error, no matter how long the larvæ may require to reach that stage. The larval period may be more than doubled by decreasing the amount of yeast. This has been repeatedly observed and corresponds to Osborne and Mendel's⁴ experiments on the retardation of growth of rats by means of incomplete food. It raises the question as to whether this is an actual prolongation of the life of the insect or merely an increase in the larval period with a corresponding decrease in the length of life of the imago. The fact that the pupal period is independent of the larval period would seem to indicate that the length of life of the imago would be independent of the time required to reach that stage in so far as the variation in the duration of the larval stage is due to lack of adequate food. Experiments are under way to determine this point. Since these experiments were started a note has appeared by Osborne, Mendel, and Ferry¹² in which they give similar indirect evidence on this point by means of the fact that the menopause in female rats is delayed by stunting. There appears to be no direct evidence, however, that there is any definite relation between the duration of the sexual life of the organism and the total life of the organism.

¹² Osborne, T. B., Mendel, L. B., and Ferry, E. L., *Science*, 1917, xlv, 294.

The most probable explanation of these results seems to be that the yeast supplies some substance or substances which are necessary for the growth of the organism and which cannot be obtained by it from protein or carbohydrate. The experiments of Loeb¹³ show conclusively that it is not a question of inorganic constituents, since this author was able to raise *Drosophila* on yeast growing on Pasteur's solution in which the salt content could be accurately controlled. These experiments also show that the yeast plant is able to synthesize the accessory substance required by the fly from simple chemical compounds of known structure. Nothing definite can be said as yet as regards the nature of the accessory substance or substances. Attempts to concentrate it by various methods of extraction, etc., have not yet led to any definite results.

It is interesting to note in this connection that Wildiers¹⁴ in 1900 came to the conclusion that some accessory substance that he called "*bios*" was needed for the growth of yeast itself but that this substance could not be synthesized by the yeast plant. A somewhat similar condition seems to exist in regard to the slime molds¹⁵ and amebæ¹⁶ which have been found to be dependent on bacteria for normal growth.

Experiments with Animal Tissues.

It seemed possible that some light might be shed on the problem if other substances could be found which were able to furnish the accessory food substances for the fly. Cultures were therefore made of various animal tissues.

The tissues were removed from the animal, ground in a mortar to a thin paste with a small amount of sand and water, the resulting suspension was poured into test-tubes with sufficient absorbent cotton to prevent the flies from drowning, and sterilized, etc., as usual. The tethelin was received in the form of a sterile powder. It was added aseptically to previously sterilized tubes containing 0.5 gm. of banana or yeast.

¹³ Loeb, J., *J. Biol. Chem.*, 1915, xxiii, 431.

¹⁴ Wildiers, E., *La Cellule*, 1901, xviii, 313. See also Amand, A., *ibid.*, 1902, xx, 225; 1904, xxi, 329.

¹⁵ Pinoy, E., *Ann. l'Inst. Pasteur*, 1907, xxi, 622, 686.

¹⁶ Biedermann, W., Winterstein's Handb. vergl. Physiol., Jena, 1911, ii, pt. i, 278.

Liver, pancreas, and kidney from the dog, and liver from the mouse were found to allow normal and perfect development, and they must therefore contain the accessory substances. Spleen, heart muscle, muscle, blood, adrenal, and thyroid from the dog were insufficient, as were muscle, testis, and tumor from the mouse. Thymus from the dog, rabbit, or calf allowed a few imagos to develop, but growth was abnormally slow, the flies were small, and many pupæ failed to hatch.

Dead sterile flies may also serve as normal food for the larvæ. This shows that the accessory substances are not broken down in the body of the fly but are preserved in an active form.

Experiments in which tethelin was added in quantities up to 60 mg. per 0.5 gm. of banana or of yeast also gave negative results; no change was noted in the growth of the larvæ in either case. The results with pancreas¹⁷ and liver¹⁸ agree with those already found for the higher animals. It is possible that the negative results in the case of some of the tissues may be due in part to the sterilization, but it is difficult to see why this should affect one tissue more than another. The larvæ grew normally on any of the tissues when they were infected with bacteria. This may explain the results of Bogdanow,¹⁹ who was unable to raise sterile meat flies (*Calliphora vomitoria*) on sterile meat (presumably muscle tissue). It seems probable that growth would have been normal on liver or pancreas and that the bacteria merely served to furnish the accessory substances which according to the present experiment are not present, in sufficient amount at least, in muscle.

SUMMARY AND CONCLUSIONS.

It has been shown in a previous paper that the fruit fly, *Drosophila*, when freed from microorganisms cannot be raised successfully on sterilized banana or on a mixture of pure proteins, sugars, salts, and fat. It is shown in the present paper that:

1. The number of flies which are able to develop on a definite quantity of yeast may be increased by the addition of banana, casein,

¹⁷ Eddy, W. H., *J. Biol. Chem.*, 1916, xxvii, 113.

¹⁸ Osborne and Mendel, *J. Biol. Chem.*, 1914, xvii, 340, 401.

¹⁹ Bogdanow, E. A., *Arch. Physiol.*, 1908. Suppl., 173.

or sugar to the yeast. These latter substances can therefore serve as food for the larvæ when supplemented by yeast.

2. The rate of growth of the larvæ is equally, or slightly more rapid on mixtures of banana and yeast containing more than 33 per cent yeast than it is on yeast alone. In mixtures containing less than this amount of yeast growth becomes slower as the amount of yeast is decreased, and finally, when the proportion of yeast is very small, becomes abnormal. Yeast therefore contains a sufficient excess of the necessary (accessory) substances to render available as food approximately twice its weight of banana.

3. Kidney, liver, and pancreas from the dog, liver from the mouse, and the bodies of the flies themselves are an adequate source of food for the larvæ.

4. Sterilized spleen, heart muscle, muscle, blood, adrenal, and thyroid from the dog are not an adequate food for the larvæ. Muscle, testis, and tumor from the mouse are also inadequate. Sterilized thymus from the dog, rabbit, or calf allow a few imagos to develop, but growth is slow and the flies are abnormally small. No effect on the rate of growth could be noted when tethelin was added to the food.

In conclusion the author wishes to acknowledge his indebtedness to Professor Jacques Loeb for many helpful suggestions during the course of the work. He is indebted to Dr. John Auer for the dog tissues, to Dr. Herbert Taylor for the mouse tissues, to Dr. Uhlenhuth for the thymus, and to Dr. T. B. Robertson for the tethelin.

THE CHEMICAL BASIS OF REGENERATION AND GEOTROPISM.

By JACQUES LOEB.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

1. It is a well-known fact that in many plants after the removal of the apex some restoration of the old form is accomplished by the growth of a hitherto dormant bud near the wound. This process has been called regeneration. It is also well known that in certain fir trees the old form is restored in such a case in an apparently different way, namely by one or more of the horizontal branches next to the apex beginning to grow vertically upwards (negative geotropism). One may wonder how it can happen that the same result, namely the restoration of the old form, is accomplished in the organic world in such different ways; and it is quite natural that occurrences of this kind should suggest to one not a mechanist the conception of mystic forces acting inside or outside the living organism towards a definite purpose, in this case the restoration of the lost apex. The writer pointed out not long ago that both phenomena, the restoration of form of a mutilated organism by geotropic bending as well as by the growing out of hitherto dormant buds may be caused by one and the same agency; namely the collection of certain chemical substances near the wound.¹ New experiments which the writer has since made seem to prove this idea to be correct.

2. In a previous paper the writer had shown that when an isolated piece of stem of *Bryophyllum calycinum*, from 10 to 15 cm. long, with one leaf attached to its apical end, is put in a horizontal position the stem will gradually bend and assume the shape of a U, with the concave side upwards and that this bending is due to the active growth of a certain layer of cells in the cortex on the lower side of the stem. When the same experiment is made with stems without a

¹ Loeb, J., SCIENCE, 1916, XLIV., 210; *Bot. Gazette*, 1917, LXIII., 25; "The Organism as a Whole," New York, 1916, p. 153.

leaf attached some geotropic bending of the stem still occurs, but at a much slower rate. From this observation the writer drew the conclusion that the leaf furnishes material to the stem which causes the growth of the cortex of the lower side of the stem, resulting in the subsequent geotropic bending of the stem.² The leaf forces this material into that part of the stem which is situated more basally than the leaf; since the part of the stem situated in front of a leaf does as a rule not show any geotropic bending. The fact that the growth leading to the geotropic curvature takes place in the cells of the lower side of a horizontally placed stem indicates that the material causing the growth collects on the lower side of the stem, which appears quite natural, since this material is a liquid, possibly containing some solid particles in suspension. A slight leakage of sap from the conducting vessels might be sufficient to account for such an accumulation of material on the under side of a horizontally placed stem.

3. Since the publication of these observations on geotropism in *Bryophyllum* the writer has been able to show that the mass of shoots which an isolated leaf can produce from its notches is a function of the mass of the leaf and that sister leaves of equal size when isolated from the stem produce equal masses of shoots under equal conditions and in equal time, even if the number of shoots produced differs considerably in the two leaves. When the mass of one set of isolated leaves is reduced by cutting out pieces from their center while their isolated sister leaves remain intact the mass of shoots produced by the two sets of sister leaves varies approximately in proportion with the mass of the leaves.³

If it is true that the geotropic bending of a horizontally placed stem depends upon the mass of material furnished to the stem by the leaf we should expect that a reduction of the mass of the leaf would correspondingly retard the rate of geotropic bending in the stem. The writer has recently carried out such experiments and they corroborate this expectation. If two sets of stems of equal length are suspended in an aquarium, each with one leaf attached to its apical end, and if the size of the leaf is reduced in one set by cutting away

² *Loc. cit.*

³ Loeb, J., SCIENCE, 1917, XLV., 436; *Bot. Gazette*, 1917 (in print).

Pieces of the leaf, the geotropic bending takes place the more slowly **the smaller** the mass of the leaf. It is difficult to conceive of a more **striking** experiment. When the mass of the leaf is reduced to zero, **the bending** is extremely slow.

4. **These** experiments suggest that the growth of the cells of a **horizontally** placed stem which gives rise to the geotropic bending is **accelerated** by substances furnished to the stem by an apical leaf; and **that** those substances might be the same as those which serve for **the** formation of roots and shoots in the isolated leaf. If this were **true**, a leaf attached to a piece of stem should form a smaller mass of shoots and roots than its sister leaf entirely detached from the **stem**, since in the former part of the material available for shoot formation should go into the stem.

It **has** been known for some time that a piece of stem inhibits the shoot formation in a leaf of *Bryophyllum calycinum*, but this inhibition **was** attributed by former writers to an influence of roots formed on **such** a piece of stem. By suitable experiments it can be shown, **however**, that the inhibition takes place also when no roots are formed on **the** stem.

It **seemed** to the writer that the inhibiting influence of the stem on the shoot production in the leaf was due, as stated, to the absorption of material from the leaf by the stem which would have served for **the** growth of roots and shoots in the leaf if the latter had been detached from the stem; and that the material flowing from the leaf into **the** stem was causing the growth of the cells in the lower side of a **horizontally** placed stem, thereby giving rise to the geotropic bending of the stem (and incidentally also to the callus formation at the base of the stem). If this were true there should exist a simple quantitative relation between the inhibiting power of the stem upon shoot formation in a leaf and the increase in the mass of the stem; namely, **the two** quantities should be approximately equal. The writer has carried out such experiments in large numbers and found that this relation holds true, namely that a piece of stem attached to a leaf increases its weight by approximately the same amount by which the shoot production in the leaf is diminished. For these experiments the following method was adopted.

5. A piece from the stem of *Bryophyllum*, containing one node

with its two leaves, is cut out from a plant and the stem split longitudinally in the middle between the two leaves, leaving one half of the stem attached to each leaf. The half stem is removed from one leaf and weighted directly. The leaf whose half stem is cut off and the leaf with a half stem still attached to it serve for the experiment. After several weeks the amount of shoots in both leaves is determined by weight and it is found that the leaf without stem had produced a larger mass of shoots than the leaf with a piece of stem attached. The latter is then removed from the leaf and weighed. It is invariably found that it has increased in weight and that this increase approximately equals the diminution in the mass of shoots in the leaf under the influence of the stem. The following may serve as an example.

Three sets of experiments were made simultaneously on 6, 7 and 7 pairs of sister leaves prepared in the way described above; one leaf was without stem and the other with one half of the split stem. The three experiments differed in regard to the length of the stem, which was in the three experiments 2 (A), 1 (B) and 0.5 cm. (C), respectively. The leaves dipped with their apices in water. The results are given in Table I. In this table we call the difference in the mass of shoots produced in the leaves *without* and *with* stems the inhibiting action of the stem. This quantity should equal approximately the sum of the mass of shoots produced in the axil of the leaf attached to the stem plus the increase in weight of the stem attached to the leaf during the duration of the experiment. The ratio of the two values should therefore approximately equal 1 (Table I.).

The experiments show that within the limit of error the mass of the stem increased in such a way as to approximately equal the inhibiting effect of the stem on shoot production in the notches of the leaf. The mass of roots produced in the leaves is neglected since it is small compared with the mass of stems.

It is almost impossible to split the living stem so perfectly that the two pieces are absolutely equal and in this way an error creeps in which can only be eliminated by a large number of experiments. In 19 different sets of experiments the leaves *without* stems produced 27.898 grams of shoots and the leaves *with* stems 9.797 grams. The inhibiting action of the stems, *i. e.*, the difference in shoot produc-

TABLE I.
Duration of Experiment 23 Days.

	Shoots Produced by Leaves		Shoots Produced by Stem		In- crease in Weight of Stems, Gm.	Inhibiting Action of Stem
	Num- ber	Weight, Gm.	Num- ber	Weight, Gm.		Increase in Weight of Stems (Including Weight of Shoots Produced by Stem)
<i>Experiment A.</i> Length of stem 2 cm. 6 pairs of sister leaves from the same plant.						
Leaves without stems.....	17	1.396				
Leaves with stems.....	5	0.266	5	0.454	0.888	<u>1.130</u> 1.342
<i>Experiment B.</i> Length of stem 1 cm. 7 pairs of sister leaves from the same plant.						
Leaves without stems.....	19	1.606				
Leaves with stems.....	13	0.823	4	0.335	0.400	<u>0.783</u> 0.735
<i>Experiment C.</i> Length of stem 0.5 cm. 7 pairs of sister leaves from the same plant.						
Leaves without stems.....	15	1.006				
Leaves with stems.....	12	0.464	4	0.105	0.289	<u>0.542</u> 0.394

tion between the leaves *without* stems and their sister leaves *with* stems was therefore 18.101 grams. According to our theory the weight of the stems which were left attached to the leaves should have increased by the same amount. The actual increase in the weight of the half stems attached to the one set of leaves was in the same time 16.695 grams. This includes the increase due to shoot production in the axil of the leaf, which was slight, amounting in all to less than 1.5 grams. The two values, 18.101 and 16.695 differ by 8.5 per cent.

It seems, therefore, probable that the inhibiting effect of the stem upon the mass of shoots produced in the leaves is due to the absorption of a corresponding quantity of material from the leaves by the stem.

6. SUMMARY AND CONCLUSIONS.

(1) The writer had shown in a former note that the mass of shoots produced in isolated sister leaves of *Bryophyllum calycinum* is in direct proportion to the masses of the leaves and that this remains true if the mass of one leaf is reduced by cutting out pieces from the center of the leaf, while the sister leaf remains intact. In this paper it is shown that the rate of geotropic bending of horizontally placed stems of *Bryophyllum calycinum*, if one apical leaf is attached to the stem, occurs at a rate increasing with the mass of the leaf. When the mass of the leaf is diminished by cutting away pieces the rate of geotropic bending is diminished also.

(2) It had been known for a long time that when a piece of stem is attached to a leaf of *Bryophyllum calycinum* the shoot production in the latter is diminished or completely inhibited. It is shown in this paper that the mass of a piece of stem attached to a leaf increases by approximately the same amount by which the shoot production in the leaf is diminished through the influence of the stem. The inference is drawn that the inhibiting effect of the stem upon shoot production in the leaf is due to the fact that the same material which would have been available for shoot production in the leaf, had the latter been detached from the stem, is now absorbed by the stem.

(3) This material gives rise in the stem to callus formation and to that growth of certain cells of the cortex which causes the geotropic bending; and if the buds of the stem are not removed it causes also shoot production on the stem. The comparatively large masses involved indicate that this material must consist chiefly of the common material required for growth, *i. e.*, water, sugars, amino acids, salts; but the accessory substances and the hypothetical specific organ-forming substances of Sachs may be included in this mass; and this is suggested by the fact that on the lower side of a horizontally placed stem, roots grow out, while shoots grow out from the upper side. There must, therefore, be associated with the material which causes geotropic bending also something which favors the growth of roots and this may be one of the hypothetical substances of Sachs.

(4) These facts give a simple explanation of the "resourcefulness" of the organism referred to in the beginning of this paper, namely that plants may restore their lost apex either by the growth of the hitherto dormant buds near the wound or by a geotropic bending of former horizontal branches next to the wound (fir trees). Our experiments suggest that the cause is the same in both cases, namely, a mass action of the nutritive, and possibly also of some specific substances, upon the cells of dormant buds or upon the cells of the lower side of horizontal branches which leads to a rapid synthesis and growth in these cells. Without the removal of the old apex this growth would not have taken place, for the simple reason that the nutritive material would have had no chance to collect near the wound in masses sufficient for the growth.

(5) The phenomena of geotropism thus turn out to be phenomena of mass action, probably of the common nutritive material circulating in the sap and they are apparently of the same nature as the growth of dormant buds, which is also due to a mass action of the same substances. Gravity need play only a passive rôle, allowing masses of liquids to "seek their level." In the literature of geotropism this phenomenon is treated as a case of "stimulation," but this treatment misses the essential point, namely, the chemical mass action involved, and it substitutes a fictitious factor, the "stimulus" of gravitation, which in all probability does not exist. The case is similar to that of heliotropism when the orientation of animals to light is treated as a "reaction to a stimulus" instead of as an instance of the photochemical law of Bunsen and Roscoe.

THE SIMILARITY OF THE ACTION OF SALTS UPON THE SWELLING OF ANIMAL MEMBRANES AND OF POWDERED COLLOIDS.

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I.

1. **W**hen dry animal membranes, like pig's bladder freed from fat by prolonged treatment with hot ether (a week or 10 days in a Soxhlet apparatus), are put into water, they will swell; they will also swell and even slightly more when put into a salt solution; but when the membrane is put first for about $\frac{1}{2}$ hour into $m/8$ solution of NaCl and subsequently into distilled water, the swelling will be twice or three times as great as in either of the other two cases. A further investigation of this problem suggested a possible connection with the structure of the membrane. An investigation of this phenomenon seemed desirable in view of the fact that Flusin¹ has tried to connect the phenomena of osmosis with the imbibition of the separating membrane.

A series of pieces of dry pig's bladder, weighing about 0.4 gm. each, were put into 50 cc. of $m/8$ NaCl, and left there for half an hour. One was put as a control into distilled water. After a half an hour each piece was freed from adhering solution by putting it between two sheets of filter paper and pressing gently; it was then weighed. The pieces which had been in $m/8$ NaCl were then put into NaCl solutions of different concentrations, namely, $m/8$, $m/32$, $m/128$, $m/512$, $m/1024$, $m/4096$, $m/8192$ NaCl, and into H_2O , and the control in H_2O was again put into H_2O . After different intervals the membranes were taken out of the solutions, freed from liquid adhering to their surface, and weighed. Table I gives the results of one such experiment.

¹ Flusin, *Ann. Chim. et Phys.*, 1908, series 8, xiii, 480.

TABLE I.

After hrs.	Swelling of dried pig's bladder in per cent of original weight of piece of bladder.								
	In NaCl								Control H ₂ O
	m/8	m/8	m/8	m/8	m/8	m/8	m/8	m/8	
$\frac{1}{2}$	187	186	190	191	184	185	173	183	149
$1\frac{1}{2}$ 16 $\frac{1}{2}$ 24	Then transferred into NaCl								
	m/8	m/32	m/128	m/512	m/1024	m/4096	m/8192	H ₂ O	H ₂ O
	204	219	289	329	310	328	301	354	165
	219	234	363	409	408	455	438	500	187
	240	242	396	464	451	510	542	562	208

These figures show the following facts: A dried membrane kept permanently in H₂O increases in weight 208 per cent in 24 hours, and when kept permanently in m/8 NaCl it increases in 24 hours slightly more, namely, 240 per cent. This was to be expected on the basis of the old experiments of Hofmeister. What the writer did not expect is the fact that if a piece of membrane is put first for 30 minutes into m/8 NaCl and subsequently for 24 hours into a weaker NaCl solution or distilled water, it swells the more the lower the concentration. Thus a membrane kept for 30 minutes in m/8 NaCl increased its weight when put subsequently into H₂O 562 per cent within 24 hours. The fact that a membrane kept permanently in m/8 NaCl swells so much less (240 per cent) must then be due to the fact that presence of the NaCl solution counteracts the swelling. We must, therefore, discriminate between two effects of the salt solution upon the swelling of a membrane, namely, *first*, a chemical reaction between the membrane and the salt, which would cause in itself a considerable swelling if it were not inhibited by the *second* effect of the solution which counteracts this tendency to swell, and the more so the higher the concentration of the salt solution. We can eliminate this second factor by allowing the membrane to react with the salt first and then transferring the membrane to H₂O after having washed off the adherent salt solution. In a recent series of papers the writer² has

² Loeb, J., *J. Biol. Chem.*, 1916, xxvii, 339, 353, 363; 1916-17, xxviii, 175.

pointed out the different behavior of the membrane of the eggs of *Fundulus* when taken directly from a salt solution or when taken from distilled water or a solution of a non-electrolyte.

2. When an attempt was made to repeat the experiment just described on pig's bladder with solid blocks of gelatine, either dry or containing varying quantities of water, they failed completely. Gelatine shows none of the peculiarities mentioned in Table I.

A 40 per cent gelatine solution was prepared and after it had set, small pieces were cut out of the jelly and exposed to air for 21 hours during which time they lost about 50 per cent in weight. Then the same experiment as represented in Table I was repeated. Table II gives the result.

TABLE II.

After	Increase in weight of blocks of gelatine in per cent of original weight.								
	In NaCl								Control H ₂ O
	m/8	m/8	m/8	m/8	m/8	m/8	m/8	m/8	
hrs. ½	23	24	27	27	25	25	30	24	19.5
	Then transferred into NaCl								
	m/8	m/32	m/128	m/512	m/1024	m/4096	m/8192	H ₂ O	H ₂ O
1½	51	52	56	54	51	52	58	50	44
19	198	193	196	190	176	176	158	170	162
24	246	236	239	231	214	216	208	214	188

The swelling in m/8 NaCl was slightly greater than in H₂O, but the gelatine first treated with m/8 NaCl and then put into H₂O for 24 hours did not swell more than the piece kept permanently in m/8 NaCl.

Thin sheets of gelatine behaved like blocks of the same material.

It was thought that the salt had perhaps not entered sufficiently into the gelatine. To avoid this possibility the gelatine was dissolved in m/8 NaCl instead of in H₂O and after setting the pieces were cut out of the gelatine. When put into NaCl solutions varying from m/8 to m/8192 or into H₂O the result was identical with the one expressed in Table II. Nor were the results different when the gelatine used was completely dried beforehand.

3. It was found that finely powdered gelatine behaved exactly like pig's bladder and behaved differently from solid blocks of gelatine. This was true when the powdered gelatine used was the same as that used for making the solid blocks mentioned in Table II.

Commercial Cooper's powdered gelatine was put through a No. 60 sieve and again through a No. 80 sieve. The grains going through the former but not through the latter sieve served for the experiment. 2 gm. of such gelatine were put into a cylindrical funnel, the bottom of which was covered with a round piece of filter paper. The upper surface of the powdered gelatine in the funnel was also covered with a round piece of filter paper in order to make it possible to pour water or salt solution on the gelatine without stirring up the particles too much. When 25 cc. of distilled water are poured on the gelatine, part of the water runs quickly through but part of the water is retained and the mass of gelatine swells. When the process is repeated, only a slight further swelling takes place, and after this no further swelling takes place no matter how much water filters through the gelatine. When instead of letting water run through the gelatine we let 25 cc. of $M/8$ NaCl run through, and repeat the process of filtering $M/8$ NaCl through the gelatine, the latter swells also and even a trifle more than in the H_2O experiment, but the maximal swelling is also soon obtained. If, however, we allow first 25 cc. of $M/8$ NaCl to run through a mass of powdered gelatine and follow this with consecutive washings by distilled water, the mass will swell considerably more with each consecutive washing than in either of the other cases.

The following experiment is the analogue of the one presented in Table I. Eight cylindrical funnels, each containing 2 gm. of powdered Cooper's gelatine (size of grain between sieves No. 60 and No. 80) were prepared in the way described above, and 25 cc. of $M/8$ NaCl were percolated through each funnel. The mass of gelatine increased in each cylindrical funnel about 18 to 20 mm. in height. After this 100 cc. of a different solution were sent through each of the eight funnels and the additional swelling was ascertained in each mass at the end of the experiment. These solutions were: $M/8$, $M/32$, $M/128$, $M/512$, $M/1024$, $M/3096$, $M/8192$ NaCl, and H_2O . The results are given in Table III.

TABLE III.

After	Swelling of powdered gelatine in mm. height of a cylinder containing the powder.								
	In NaCl								Control H ₂ O
	m/8	m/8	m/8	m/8	m/8	m/8	m/8	m/8	
$\frac{1}{2}$ hr.	19.5	20	20	19	17	18	18.5	20	15
Additional swelling..	Then 100 cc. of the following solutions were allowed to run through.								
	NaCl							H ₂ O	H ₂ O
	m/8	m/32	m/128	m/512	m/1024	m/4096	m/8192		
	8(?)	5	5	12	21	31	40	64	14

The increase in swelling is expressed in terms of the height of the cylindrical mass of gelatine.

It is obvious that when powdered gelatine is treated first with m/8 NaCl and subsequently with H₂O it retains much more water than when it is perfused permanently with H₂O or permanently with m/8 NaCl. This can also be explained on the assumption that the phenomenon is due to two different effects, first the reaction between salt and gelatine which increases the swelling, and second the inhibition of the swelling if the gelatine is washed in the salt solution. This inhibition reminds us of the inhibition of acid swelling of gelatine by the presence of salts, though the mechanism may be different in the two cases.

From these experiments it is obvious that pig's bladder and powdered gelatine have a peculiarity in common which we were not able to discover in solid blocks of gelatine, namely, to swell considerably more when a short treatment with m/8 NaCl is followed by a treatment with H₂O, than when the mass is treated exclusively with H₂O or exclusively with salt. The property which powdered gelatine and pig's bladder have in common is that they consist of very small discrete particles, grains in the one and fibers in the other; while the gelatine in a block must be considered as a homogeneous mass or as one enormous particle. It should also be stated that this peculiar behavior of powdered gelatine is probably found in many powdered colloids; thus powdered

ovomucoid kindly given to us by Dr. López-Suárez behaved exactly like powdered gelatine.

To make the demonstration complete we should add that the after effect of a previous salt treatment just described is found also if other concentrations of NaCl than $M/8$ are used, namely, $M/4$, $M/2$, $M/1$, etc., but that the after effect ceases when the NaCl concentration is too low, namely, below $M/64$ or $M/128$ NaCl.

It should also be said that if we leave powdered colloids or pig's bladder permanently in a salt solution nothing comparable to this after effect is noticed even if the NaCl solution is very weak. In such cases the maximum swelling is soon reached. Table IV gives the results of such an experiment with powdered gelatine, which covers also the case for pig's bladder. We have mentioned the fact

TABLE IV.

	Swelling of powdered gelatine in mm. height of a cylinder.											H ₂ O
	NaCl											
	2 M	M/1	M/2	M/4	M/8	M/16	M/32	M/64	M/128	M/256	M/512	
After first 25 cc. of solution.....	23	22	21	24	22	20	21	21	20	20	21	19
Additional swelling after further 25 cc. of solution.....	11	8	6	4	4	2	2.5	2	2	2	2	2
Additional swelling after third 25 cc. of solution.....	0	0	0	0	0	0	0	0	0	0	0	0

that if only $M/8$ NaCl solutions are allowed to percolate through powdered gelatine the maximum swelling is soon reached and no further swelling takes place. This is true for all concentrations of NaCl tried.

We only notice a slightly increased effect with the increase of the concentration.

II.

The Effect of Different Ions.

1. The excessive swelling, observed in pig's bladder, in powdered gelatine, or in ovomucoid when a short treatment with NaCl solution of not too low a concentration is followed by a treatment with distilled water, is not confined to NaCl, but is produced by many if not all neutral salts with a univalent cation; while the neutral salts with bivalent cations have no such effect. Pieces of pig's bladder were put for 30 minutes into $M/8$ LiCl, NaCl, KCl, $MgCl_2$, $CaCl_2$, and $SrCl_2$. Table V gives the result.

TABLE V.

After	Increase in weight of pig's bladder in per cent of original weight in $M/8$ solutions of:						
	LiCl	NaCl	KCl	$MgCl_2$	$CaCl_2$	$SrCl_2$	Control H_2O
hrs. $\frac{1}{2}$	181	193	186	225	164	162	139
	They were then transferred to distilled water.						
$\frac{1}{2}$	306	335	325	169	138	149	145
4	592	547	454	189	138	161	151
22 $\frac{1}{2}$	662	598	434	195	134	165	145

Leaving aside minor differences, it is obvious that the previous treatment of the membrane with Li, Na, and K causes a considerable increase in swelling, while this increase is lacking in the case of Mg, Ca, and Sr. The salts of the alkali earth simply prevent the subsequent increase in the swelling caused by NaCl or LiCl. Mg is less active than Ca or Sr, which was to be expected. *It cannot be said that $CaCl_2$ or $SrCl_2$ affect the swelling in the opposite way from that caused by NaCl, since the effect of $CaCl_2$ does not differ much from that of distilled water.*

All salts of Na cause the after effect though the quantity of swelling varies with different anions (Table VI).

TABLE VI.

After	Swelling of pig's bladder in per cent of original weight in solutions of different sodium salts.							
	M/8 NaCl	M/8 NaNO ₃	M/8 NaCH ₃ COO	M/8 Na ₂ SO ₄	M/16 Na ₂ SO ₄	M/8 Na ₂ tartrate	M/16 Na ₂ tartrate	Control H ₂ O
hrs. ½	188	209	157	195	222	172	201	167
	They were then transferred to distilled water.							
½	325	312	290	345	464	336	418	189
5	638	464	505	644	846	644	872	221
22½	758	840	490	659	832	1,071	1,400	223

The experiment shows clearly that a short treatment of the membrane with any sodium salt causes a considerable further swelling in H₂O after the free salt solution is leached out. One fact, however, stands out, namely, that the striking difference between the action of univalent and bivalent cations is in no way repeated among the anions. This is in harmony with the writer's first extensive experiments on antagonistic salt action on *Fundulus* in which he showed that the toxic action of high concentrations of salts with univalent cations could be inhibited by small quantities of salts with a bivalent cation, while no such valency effect could be found for the anions.*

2. The same difference in the effect of the salts with univalent and bivalent cations upon the subsequent swelling of pig's bladder in distilled water can be found in powdered gelatine. 2 gm. of powdered gelatine (of grain size 60-80) were put into each of a series of cylindrical funnels, and 25 cc. of M/8 LiCl, NaCl, KCl, MgCl₂, CaCl₂, SrCl₂, and BaCl₂ were kept in contact with the gelatine for half an hour and then allowed to run off. Four times in succession 25 cc. of distilled water were then allowed to run through each cylinder. Table VII gives the swelling in mm. height of the cylinder.

The amount of swelling in the different salt solutions was too small to be discovered. In the after effect, however, the striking difference between the salts with univalent and bivalent cations (which was also noticeable in the case of the swelling of pig's membrane) shows

* Loeb, *Arch. ges. Physiol.*, 1901, lxxxviii, 68; *Am. J. Physiol.*, 1901-02, vi, 411.

TABLE VII.

	Swelling in mm. height of cylinder of powdered gelatine under the influence of different chlorides.							
	m/8 LiCl	m/8 NaCl	m/8 KCl	m/8 MgCl ₂	m/8 CaCl ₂	m/8 SrCl ₂	m/8 BaCl ₂	Control H ₂ O
Swelling under influence of 25 cc. salt solution.....	23	25	24	24	24	24.5	24	24
Additional swelling under influence of 100 cc. H ₂ O (four washings).....	15	33	25	4.5	4	3.5	2.5	5.5

itself. There is practically no difference in the after effect of CaCl₂ upon the swelling of the membrane in distilled water and the swelling caused in distilled water without any previous salt treatment.

3. No difference of this kind in the after effect of a treatment with NaCl and CaCl₂ could be discovered in solid blocks or sheets of gelatine.

III.

Antagonistic Salt Action.

1. In 1901³ the writer showed that the injurious effects which a salt with univalent cation has upon the eggs of *Fundulus*, as soon as the concentration of the salt exceeds a certain concentration, can be inhibited by the addition of a very small quantity of a salt with a bivalent cation; while the addition of a salt with a bivalent anion had no such effect. The writer drew from these facts the conclusion that the antagonistic action of the bivalent cation in this case must be due to an action of the salts upon the state of colloids, but for a long time it was found difficult to imitate such an antagonistic salt action directly on colloids. Recently analogies have been shown to exist by Schryver⁴ on gels of cholate solutions, by Clowes for soaps,⁵ by Lenk⁶

³ Schryver, S. B., *Proc. Roy. Soc., Series B*, 1914, lxxxvii, 366; 1916, lxxxix, 176. Schryver, S. B., and Hewlett, N., *ibid.*, 1916, lxxxix, 361.

⁵ Clowes, G. H. A., *J. Phys. Chem.*, 1916, xx, 407; *Science*, 1916, xliii, 750.

⁶ Lenk, E., *Biochem. Z.*, 1916, lxxiii, 15, 58.

for the swelling of gelatine, and by Fenn⁷ for the precipitation of dissolved gelatine by alcohol in the presence of different salts.

A very striking antagonistic salt action can be demonstrated in the case of the after effect of NaCl upon the subsequent swelling of pig's bladder and powdered colloids in distilled water. This swelling is inhibited if a comparatively small quantity of CaCl_2 (or any other salt with bivalent cations) is added to the NaCl.

The experiment consisted in the following. Pieces of dry pig's bladder were put for 30 minutes into the following solutions:

50 cc. M/8 NaCl	
50 " M/4 " + 1 cc. M/8 CaCl_2 + 49 cc. H_2O	
50 " M/4 " + 2 " M/8 " + 48 " "	
50 " M/4 " + 4 " M/8 " + 46 " "	
50 " M/4 " + 8 " M/8 " + 42 " "	
50 " M/4 " + 16 " M/8 " + 34 " "	
50 " M/4 " + 32 " M/8 " + 18 " "	

As the reader will see, each of these solutions was M/8 in regard to NaCl but contained increasing concentrations of CaCl_2 . After the membranes had been in the solutions for 30 minutes they were transferred to distilled water. It will be seen that the membrane previously treated with pure NaCl swelled considerably while the ones treated previously with NaCl + CaCl_2 swelled the less the more CaCl_2 was added.

TABLE VIII.

After	Antagonism between NaCl and CaCl ₂ on the subsequent swelling of pig's bladder when put into distilled water. Increase in weight in per cent of original weight.							
	Cc. m/8 CaCl ₂ in 100 cc. m/8 NaCl							Control H ₂ O
	0	1	2	4	8	16	32	
hrs. ½	189	173	182	185	157	159	164	143
The membranes were then all transferred to distilled water.								
3	336	305	339	280	155	155	160	145
21	345	345	(430(?))	269	137	154	167	

⁷ Fenn, W. O., *Proc. Nat. Acad. Sc.*, 1916, ii, 534, 539. In one of the examples mentioned by Fenn, *e.g.*, the precipitation of gelatine in mixtures of solutions of Na_3 citrate and CaCl_2 by alcohol, the precipitation depends to a large extent upon the formation of a supersaturated solution of calcium citrate which is precipitated by alcohol even if no gelatine is present.

It is obvious that the addition of 4 cc. of $M/8$ CaCl_2 to 100 cc. of $M/8$ NaCl already inhibits markedly the subsequent swelling of the membrane in distilled water and that the addition of 8 cc. of $M/8$ CaCl_2 to 100 cc. of $M/8$ NaCl inhibits this swelling almost completely.

If the reader will look over the first row of figures representing the swelling of the membranes while *in* the salt solutions the antagonistic salt action will be seen to be extremely small if it exists at all.

It should also be pointed out once more that the antagonistic action between NaCl and CaCl_2 is not the algebraic mean between a swelling effect of NaCl and a dehydrating effect of CaCl_2 . Table V has already contradicted such an assumption. In membranes not treated previously with any salt the swelling in H_2O amounted in Table VIII to 145 per cent, while in membranes which had been treated for 30 minutes with 100 cc. of $M/8$ NaCl + 32 cc. of $M/8$ CaCl_2 and were then transferred to H_2O it amounted to 167 per cent. The addition of CaCl_2 served only to prevent the enormous increase in weight which a previous treatment with $M/8$ NaCl alone induces, namely, in this experiment 345 per cent, but it cannot be said that CaCl_2 and NaCl affect the membrane in an opposite sense.

2. The antagonism experiment just described is successful also with powdered gelatine. 2 gm. of powdered gelatine (size of grain 60–80) were put into a series of funnels as (described in the beginning of this paper) and first 25 cc. of $M/8$ NaCl with different quantities of CaCl_2 (as described in the experiment with pig's bladder) were allowed to run through the gelatine.

Subsequently 75 cc. of distilled water were allowed to run through each cylinder. The result was striking, inasmuch as *in* the salt solution the swelling was approximately the same in pure NaCl and in NaCl with CaCl_2 ; but in the subsequent treatment with H_2O the swelling was enormous in the powdered gelatine treated previously with pure $M/8$ NaCl , while this after effect was prevented when 8 cc. or more of CaCl_2 were added to 100 cc. $M/8$ NaCl (Table IX).

Mg and Sr act similarly to CaCl_2 .

The same antagonistic effect was observed with powdered ovomucoid.

3. The writer has not been able to find such an antagonistic action in the after effect of a previous treatment of solid blocks of gelatine

with mixtures of NaCl and CaCl_2 . The experiment in Table X was performed with solid pieces of dry gelatine.

While in the salt solution the swelling was greater in both $\text{m}/8$ NaCl and $\text{m}/8$ CaCl_2 than in pure distilled water, in the subsequent treatment with distilled water no after effect of the nature of that found in powdered gelatine or in pig's bladder could be discovered.

TABLE IX.

	Swelling of powdered gelatine in mm. height of a cylinder.							
	Cc. $\text{m}/8$ CaCl_2 in 100 cc. $\text{m}/8$ NaCl							Control H_2O
	0	1	2	4	8	16	32	
After 25 cc. salt solution had been added.....	22	20	23	22	23	21	21	19
Then 75 cc. distilled water were allowed to filter through.								
Additional swelling after the 75 cc. distilled water had percolated.....	30	37	29	20	3.5	3.5	5	3

TABLE X.

After	Increase in weight of solid blocks of dry gelatine in per cent of the original weight of the solution.							
	Cc. $\text{m}/8$ CaCl_2 in 100 cc. $\text{m}/8$ NaCl						Controls.	
	0	1	2	4	8	16	100 cc. $\text{m}/8$ CaCl_2	H_2O
hrs. $\frac{1}{2}$	101	108	99	106	113	99	110	96
The pieces of gelatine were then transferred to distilled water.								
3	245	265	243	264	265	241	267	257
23	483	550	469	514	524	500	527	538

This difference in behavior between powdered gelatine and gelatine in a solid block cannot be ascribed to a difference in the chemical nature of the gelatine used in both cases since the results were the same when the blocks of gelatine were made by dissolving Cooper's powdered gelatine in warm water and allowing the solution to set. The results were also the same when the solid blocks of gelatine were only half dry at the beginning of the experiment, or when they were exposed to the salt solution longer or less than 30 minutes.

IV.

The Influence of the Size of the Particles of Powdered Gelatine upon the Salt Action.

The fact that the after effect of a treatment with neutral salts with monovalent cation is the same for pig's bladder and for powdered gelatine, while it is different for gelatine in the form of one solid block, suggests that we are dealing with a surface effect of the salt. In order to test this suggestion powdered gelatine of four different sizes of particles was prepared, by sifting Cooper's powdered gelatine through sieves with different openings, namely, with 50, 60, 80, 100, and 120 wires per inch. The first lot contained particles which went through 50, but not through 60 (designated 50-60); the next those going through Sieve 60 but not through No. 80 (designated 60-80), and so on. 2 gm. of each of these particles were put into a funnel as described and first 25 cc. of $M/8$ NaCl were poured on each lot. In order to insure equal action of the salt the solution was kept in contact with the gelatine for $\frac{1}{2}$ hour before it was allowed to filter. The swelling was measured and then 25 cc. of distilled water were poured on the gelatine and the H_2O was kept in contact with the gelatine for 20 minutes before it was allowed to filter through, and the swelling was measured again. This was followed by again pouring 25 cc. of distilled water into each funnel, keeping the H_2O in contact with the gelatine for 45 minutes, then allowing it to run through, and then again measuring the swelling. Table XI gives the result.

TABLE XI.

	Swelling of powdered gelatine of different grain size. Swelling measured in mm. height of the cylindrical mass of gelatine.			
	Size of particles.			
	50-60	60-80	80-100	100-120
Swelling after 25 cc. $M/8$ NaCl had been allowed to percolate.	23	25	24	23
Additional swelling after first 25 cc. H_2O had been allowed to percolate.	3	6	8.5	10
Additional swelling after the second 25 cc. H_2O had been allowed to percolate.	19	27	31	38

It is obvious that the smaller the particles the greater the retention of water and the greater the swelling. This would be expected if the after effect of the salt upon the swelling in distilled water were a surface effect; for the total surface of a given mass of gelatine is of course the greater the smaller the size of the particles.

It should be possible to calculate the increase in surface with the decrease of the size of the powdered granules if the phenomenon were not complicated by a second variable which acts in the opposite sense as the size of the granules and which the writer believes to be the process of packing. The "packing" diminishes the free area of the particles.

The excessive swelling of pig's bladder under the influence of a previous treatment of NaCl is therefore a phenomenon which can be repeated in powdered gelatine but not in solid blocks of gelatine, and hence must be due to a difference in the structure of the two groups of systems, the gelatine block being homogeneous with only a comparatively small outer surface while the pig's bladder and the mass of powdered gelatine or ovomucoid consist of small discrete elements with an enormous internal surface.

The writer is inclined to believe that the salts combine with the gelatine and as a consequence modify the chemical affinity of the surface of the discrete particles for water. The result is a greater retention of water after the free salt solution has been replaced by distilled water. The gelatine or ovomucoid salts with univalent cations retain the water in such cases very powerfully, while the colloidal Ca salts do not possess this peculiarity. The mechanism of the swelling of powdered colloids or animal membranes like pig's bladder in distilled water, after a previous treatment with a neutral salt with univalent cation, is different from the mechanism of swelling of a solid block of gelatine under the influence of acid or alkali. The latter case has been explained very elegantly by Procter.⁸

⁸ Procter, H. R., *J. Chem. Soc.*, 1914, cv, 313. Procter, H. R., and Wilson, J. A., *ibid.*, 1916, cix, 307.

V.

The Antagonistic Salt Action on the Percolation of Water through Powdered Colloids.

In 1905 the writer⁹ suggested that the antagonism between salts with monovalent and bivalent cations was due to the fact that small quantities of bivalent cations prevented the diffusion of the salts with univalent cations through the animal membranes. This idea has since been generally accepted and has received support by the work of many experimenters, especially the brilliant experiments of Osterhout¹⁰ on *Laminaria*. It seemed, therefore, of interest to see in which sense the salts with univalent and bivalent cations influence the rate of diffusion or percolation of water through powdered colloids.

We must keep in mind that the antagonistic salt effects described in this paper differ in an essential point from the antagonistic salt effects on the living organism. The observations on the antagonistic action of salts on living organisms were all made while the living object was *in* the salt solution; and the only exceptions from this rule are the observations on washed eggs.² The antagonistic effects described in this paper deal with the behavior of colloids *after* the salt solution has been replaced by distilled water and after all the free salt solution has been washed away.

It is very easy to examine the influence of a salt treatment upon the rate of percolation of liquids through powdered colloids. 2 gm. of powdered gelatine or ovomucoid are put into a cylindrical funnel in the way described at the beginning and 25 cc. of a salt solution carefully poured on top of the mass. This solution runs through very rapidly as long as the particles are not too small. This is followed by pouring 25 cc. of distilled water upon the mass and this is then repeated. The rate of percolation becomes slower with each washing (due possibly to a denser packing of the particles) and after two or three washings with distilled water a definite effect of the previous salt treatment upon the rate of percolation can be discovered. This effect is exactly the reverse of the influence of the salts upon the sub-

⁹ Loeb, *Arch. ges. Physiol.*, 1905, cvii, 252.

¹⁰ Osterhout, W. J. V., *Plant World*, 1913, xvi, 129; *Proc. Am. Phil. Soc.*, 1916, lv, 533.

sequent swelling in distilled water. Thus a treatment of powdered gelatine with $m/8$ NaCl increases the rate of swelling of the mass in distilled water (after the salt solution is washed off) but it diminishes the rate of percolation of distilled water through the mass. $CaCl_2$ neither favors swelling nor does it retard the rate of percolation; it may accelerate it slightly.

It is thus easy to demonstrate an antagonistic salt action upon the rate of percolation of water through powdered gelatine. The same solutions as in Table VIII were used in the following experiment. 1 gm. of powdered gelatine was put into each funnel and at first the various mixtures of NaCl + $CaCl_2$ solutions (25 cc. in each case) were allowed to run through. This was followed by repeated washings with 25 cc. of distilled water. Table XII gives the cc. of water which percolated from the funnels into a measuring cylinder after 25 cc. of H_2O had been poured on the mass for the third time (third washing).

TABLE XII.

Cc. of H_2O which percolated in	Rate of percolation of 25 cc. of distilled water through powdered gelatine after a previous treatment with the following solutions and two washings with 25 cc. H_2O							
	Cc. $m/8$ $CaCl_2$ in 100 cc. $m/8$ NaCl							H_2O
	0	1	2	4	8	16	32	
159 min.	9	10.4	13.6	13.2	19.1	18.4	21.4	22.4
17 hrs.	23	24	26*	24	30*	26.5	25.5	27*

* Some of the water retained in previous washings had filtered through.

The rate of percolation is slowest in the gelatine previously treated with $m/8$ NaCl; and here the swelling is greatest. This is natural since the swelling as well as the lowering of the rate of percolation have the same cause, namely, the retention of water by the powdered gelatine. The same experiment can be made with powdered ovomucoid.

The writer has not yet tried any experiments on the influence of a previous salt treatment on the diffusion of water through a membrane of pig's bladder, though he intends to do so.

The experiments mentioned here bear a certain resemblance to the

observations of soil chemists on the percolation of water through soil previously treated with salts. The writer's attention was called to this work by Professor Lipman in Berkeley, in whose laboratory the subject has recently been investigated by Mr. Sharp.¹¹ It seems that many years ago A. Meyer first observed the fact that if soil had been soaked with certain salts it became impermeable for water, after the salt had been leached out. Schlösing and Van Bemmelen showed that the phenomenon was connected with a greater degree of suspensibility of the soil after such a treatment.¹² Soil treated with $m/8$ NaCl becomes almost impermeable for water after the salt solution is washed out. No measurable swelling of the soil follows when the soil is first treated with $m/8$ NaCl, then with distilled water until all the salt solution is driven out, and the soil becomes highly impermeable. The impermeability in this case is much greater than in the case of powdered gelatine or ovomucoid and the suspicion is justified that the impermeability of the soil after a treatment with NaCl is at least partly due to a denser packing of the particles. This variable may also be at least partly responsible for the retardation of percolation of water through powdered gelatine after a previous treatment with NaCl.

CaCl_2 does not retard the subsequent percolation of water through soil and it is easy to demonstrate the antagonism between NaCl and CaCl_2 upon the subsequent rate of percolation of water through soil after the salt is leached out.

10 gm. of finely powdered garden soil were put into each of a series of cylindrical funnels. Then 25 cc. of the antagonistic salt mixtures were poured on the soil and the time measured until 20 cc. of the solution had diffused into a measuring cylinder put under the funnel. Then 25 cc. of H_2O were poured into each funnel and again the time for 20 cc. of liquid to run through the soil was measured, and this was repeated three times. Table XIII gives the results.

It may be possible to make practical use of this action of Ca (which seems to be the same for all bivalent cations) for rendering impermeable soil permeable for water.

¹¹ Sharp, L. T., *Proc. Nat. Acad. Sc.*, 1915, i, 563; *Univ. Cal. Publ. in Agricult. Sc.*, 1916, i, 291.

¹² Van Bemmelen, J. M., *J. prakt. Chem.*, 1881, xxiii, 388.

TABLE XIII.

	Time in minutes for 20 cc. of liquid to run through 10 gm. of soil in a cylindrical funnel.							Control H ₂ O
	Cc. m/8 CaCl ₂ in 100 cc. m/8 NaCl							
	0	1	2	.	8	16	32	
25 cc. salt.	34	37.5	35	30.5	33	33	34	43
25 " H ₂ O.	73.5	66	50	46.5	40	34	33.5	44
25 " "	1,185*	1,185	1,185	1,185		66.5	43	68.5

* *I.e.*, over night.

The writer does not wish to enter into the cause of this behavior of soil beyond mentioning that if all organic matter of the soil is destroyed by ignition a treatment of such soil with NaCl will no longer call forth the striking inhibition of the percolation of H₂O after the salt is leached out; but that an addition of some finely powdered organic colloid (powdered dry oak leaves, gum tragacanth, powdered gelatine, or ovomucoid) can restore to some extent this effect of a previous washing with NaCl. A mixture of finely powdered marble and powdered colloids acts like a mixture of ignited soil and organic colloids.

SUMMARY OF RESULTS.

1. Dried pig's bladder, freed from fat, when treated for a short time with a solution of a salt with univalent cation swells considerably more when subsequently put into distilled water, than it does if it remains permanently in the same salt solution or when it remains permanently in distilled water without a previous salt treatment.

2. It is assumed that this increased swelling of the membrane in distilled water after a previous treatment with one of the salts with univalent cation is due to an interaction between the salt and a constituent (probably protein) of the membrane; when the bladder remains permanently in the salt solution the latter prevents the swelling which takes place as soon as the salt solution is replaced by H₂O or a very weak salt solution.

3. A treatment of the membrane with salts with a bivalent cation (Mg, Ca, Sr, and Ba) does not induce the excessive swelling when the membrane is subsequently exposed to distilled water. Neither

does such a treatment induce a dehydration of the membrane. Membranes previously treated with salts with a bivalent cation swell when afterwards put into distilled water approximately to the same extent as membranes that have not been treated with any salt.

4. The addition of about 8 cc. of $\text{m}/8 \text{ CaCl}_2$ to 100 cc. of $\text{m}/8 \text{ NaCl}$ prevents the after effect which a treatment with a pure $\text{m}/8 \text{ NaCl}$ solution produces. It should be noticed that CaCl_2 does not influence swelling in the opposite sense from that of NaCl , but that it renders the after effect of the treatment with NaCl impossible in some other way.

5. It is impossible to repeat these effects of a previous salt treatment upon the subsequent swelling in distilled water with solid blocks of gelatine, or with sheets of gelatine.

6. It is, however, possible to repeat them with powdered gelatine or with powdered water-insoluble ovomucoid (and probably a large number of other powdered colloids).

7. The fact that pig's bladder behaves in regard to these phenomena like powdered colloids but not like solid blocks or sheets of gelatine suggests that the salt effects described in this paper are due to an action upon the surface of colloidal particles (fibers in the case of pig's bladder).

8. This suggestion is supported by the fact that the effect of a previous treatment with $\text{m}/8 \text{ NaCl}$ upon the subsequent swelling of a given mass of powdered gelatine in distilled water is greater when the size of the particles is smaller and hence the total internal surface greater.

9. It follows from all this that the mechanism of the swelling described in this paper is of a different nature from that observed in solid masses of gelatine under the influence of acid or alkali.

10. Observations upon the rate of percolation of water show that the effect of salt upon the subsequent rate of percolation of distilled water through the powdered gelatine varies inversely with the rate of swelling. A previous treatment with $\text{m}/8 \text{ NaCl}$ solution retards the percolation of water through the powdered gelatine, while a previous treatment of the mass with $\text{m}/8 \text{ CaCl}_2$ has no such effect. The addition of a small quantity of CaCl_2 to NaCl prevents the subsequent retardation of the rate of percolation of water as it prevents the swelling.

11. It has been known that a treatment of soil with NaCl renders the soil almost impermeable to water after the salt is leached out. In this case, however, no swelling of the soil seems to take place and the writer is not certain whether the influence of a salt treatment upon the percolation of water through powdered gelatine and ovomucoid is identical with or only analogous to that upon the percolation of water through soil.

STUDIES OF ACIDOSIS.

I. THE BICARBONATE CONCENTRATION OF THE BLOOD PLASMA, ITS SIGNIFICANCE, AND ITS DETERMINATION AS A MEASURE OF ACIDOSIS.*

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* A preliminary report was published in *Proc. Soc. Exp. Biol. and Med.*, 1915.

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I. Blood Bicarbonate and Acidosis.

Free carbonic acid is present in the body fluids in such concentration that it automatically converts into bicarbonate all bases not bound by other acids. *The bicarbonate therefore represents the excess of base* which is left after all the non-volatile acids have been neutralized and is available for the immediate neutralization of further acids. In this sense it constitutes the *alkaline reserve of the body*. The bicarbonate concentration of the blood is representative of that of the body fluids in general, and is normally maintained at a definite level. Entrance of free acids reduces it to an extent proportional to the amount of the invading acid.

While in data to be published in this and subsequent papers we believe that we have broadened the foundation of facts on which the above statements stand, the latter are either contained in the propositions laid down by Henderson (1908, *b*; 1909, *b*), as the result of observations by himself and others,¹ or are self-evident corollaries of those propositions. They establish the blood bicarbonate as a criterion of the acid-base balance of the body. Accordingly, for use in the present series of papers, we define *acidosis* as a condition in which the concentration of bicarbonate in the blood is reduced below the normal level. The definition appears a necessary preliminary because of present confusion in the literature, different authors regarding acidosis differently as "acid intoxication," as a condition in which acetone bodies are formed, or as an actual increase in the hydrogen ion concentration of the blood.

Acidosis in the sense defined may result, as in diabetes, from such an overwhelmingly rapid production of acids that even an apparently undamaged eliminating mechanism working at several times the usual rate cannot dispose of them. Or it may result, as in nephritis, from inability to eliminate acids even at the moderate rate at which normal metabolism produces them. In either case the retained acid decomposes body bicarbonate, forming in its place the salt of the invading acid.

¹ Henderson's monograph and the papers by Henderson and Palmer contain so complete an exposition of the mechanism by which phosphates and the kidneys assist in maintaining body neutrality that this portion of the subject is given minimum consideration in the present paper.

The bicarbonate not only represents the alkaline reserve of the body, but its normal concentration in the blood is so definite that it constitutes a physiological constant. The blood plasma of the normal adult contains 50 to 65 per cent of its volume of CO_2 gas bound as bicarbonate. The limits of variation are similar in magnitude to those of the pulse rate. By utilizing as a standard the normal bicarbonate concentration we can reduce the term "acidosis" to as definite a meaning as "fever" or "tachycardia." In each case a condition is indicated in which one of the physiological constants falls or rises to an abnormal level. The possible causes are numerous, but the result, in the case of acidosis a lowering of the blood bicarbonate, is an accurately definable and determinable phenomenon. Like accelerated pulse rate or increased temperature, it may occur temporarily even in health, *e.g.*, as the result of muscular exertion and the consequent lactic acid formation (Christiansen, Douglas, and Haldane, 1914). It is not necessarily a pathological condition in itself, but is a symptom of disturbed function. Like fever or tachycardia, however, acidosis in itself becomes a danger when it has reached a sufficient degree of intensity.

The hydrogen ion concentration, C_H , of the blood is a physiological constant even less variable than the plasma bicarbonate (Lundsgaard, 1912), the normal value of approximately $10^{-7.45}$ being maintained with the utmost tenacity by the normal organism. Nevertheless, as a standard for measuring changes in the acid-base balance, it appears less desirable than the bicarbonate, for the reason that, while the bicarbonate decreases progressively as soon as the normal excess of bases over acids begins to be depleted, rise of the blood C_H is usually one of the latest changes that follow.

Benedict (1906) and Michaelis (1914, p. 105), for example, have observed in diabetic acidosis an increased C_H only after terminal coma had set in, and Peabody (1914) has made similar observations in the acidosis of cardiorenal disease. In both types of cases coma occurs only after the blood bicarbonate has been reduced to a fraction of its normal value.

The reason for the lateness of the stage at which increase in blood C_H appears is the fact that, until a large part of the bicarbonate has been exhausted, the organism can, by accelerated respiration, main-

tain the ratio² $\frac{H_2CO_3}{NaHCO_3}$ in the arterial blood at its normal value. And the C_H , being directly proportional to this ratio, is thereby also kept normal.

The manner in which the body uses carbonic acid and bicarbonate in order to maintain its neutrality has been most clearly described by Henderson in his monograph (1909, *b*). The normal C_H is maintained by the mechanism for keeping the $\frac{H_2CO_3}{NaHCO_3}$ constant. From the law of mass action

$$C_H = K \frac{H_2CO_3}{CO_2} = K \frac{H_2CO_3}{\lambda NaHCO_3}$$

λ being the degree of dissociation of $NaHCO_3$ into Na^+ and HCO_3^- in the blood, and K the ionization constant of H_2CO_3 . Since λ varies but slightly within the range of conditions encountered within the blood plasma, one may state that in the plasma *the hydrogen ion concentration varies directly as the value of the molecular ratio* $\frac{H_2CO_3}{NaHCO_3}$. Hasselbalch (1916, *b*) has shown that this law holds so

accurately that he regards the determination of the $\frac{H_2CO_3}{NaHCO_3}$ ratio as an even more reliable means than the gas chain for determining blood hydrogen ion concentration. Whenever, either by increased rate of CO_2 production or by decomposition of $NaHCO_3$ by acid, the ratio $\frac{H_2CO_3}{NaHCO_3}$ is increased, the C_H of the blood is proportionately increased, and stimulates respiration. More rapid ventilation follows until the H_2CO_3 of the blood is so reduced that the normal $\frac{H_2CO_3}{NaHCO_3}$ ratio, and consequently the normal C_H , is restored.

The respiratory response is so sensitive to this stimulus that Campbell, Douglas, Haldane, and Hobson (1913) observed that an increase of only 1 mm. in the CO_2 tension accelerated the rate of ventilation 60 per cent, and Boothby (1915) has observed that the heart output is similarly increased in the effort to rid the body of excess CO_2 .

We find that plasma, obtained by drawing blood from the arm vein and centrifuging at once, contains at 37° and normal CO_2 tension approximately 60 volume per cent of CO_2 gas bound as bicar-

² A minor portion of the combined carbonic acid of the plasma is, of course, neutralized by bases other than sodium; but as the acid-neutralizing power of the strong bases does not differ greatly we follow Henderson's convenient practice of using " $NaHCO_3$ " to indicate the total bicarbonate.

bonate. The concentration of CO_2 in the form of H_2CO_3 calculated from the average arterial CO_2 tension of 42 mm. is 3 volume per cent $\left(\frac{42}{760} \times 100 \times 0.54 = 3.0\right)$, 0.54 being the solubility coefficient of CO_2 in blood plasma at body temperature, as determined by Bohr). Consequently the normal ratio

$$\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3} = \frac{3}{60} = \frac{1}{20}$$

a value which agrees approximately with that calculated from the known values of the constants in the equation³

$$\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3} = \frac{\lambda C_{\text{H}^+}}{K}$$

The process of accelerating ventilation and circulation in proportion to the fall in plasma bicarbonate, so that the ratio $\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}$ and the resulting C_{H^+} are kept constant, can apparently continue until acidosis is so intense that the respiratory and circulatory mechanisms are no longer able to eliminate carbonic acid so rapidly as to keep its concentration down to one-twentieth that of the depleted bicarbonate. The level to which the bicarbonate falls before this failure of compensation occurs must vary with the sensitiveness of the nervous control and the efficiency of the respiratory and circulatory mechanisms, and has never been definitely fixed, although in diabetes and nephritis it appears to be a small fraction of the normal (Michaelis, 1914, p. 105; Peabody, 1914).

To distinguish the stage of acidosis in which the respiratory mechanism no longer keeps the carbonic acid concentration of the arterial blood down to the normal fraction of approximately one-twentieth the bicarbonate, and in which consequently the C_{H^+} actually does increase, Hasselbalch and Gammeltoft (1915) have already used the

³ The average C_{H^+} is approximately 0.35×10^{-7} . According to Michaelis and Rona (1912), $K = 4.4 \times 10^{-7}$, λ for blood conditions = 0.605. From these constants,

$$\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3} = \frac{0.605 \times 0.35 \times 10^{-7}}{4.4 \times 10^{-7}} = \frac{1}{21}$$

term "*uncompensated acidosis*," which seems well worth general adoption. So long, on the other hand, as the respiration, despite decreased bicarbonate, succeeds in keeping down to normal limits the $\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}$ ratio, and consequently the C_{H^+} , the condition is one of *compensated acidosis*. We shall in future use this nomenclature.

A maintenance of the $\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}$ ratio at a constant value can, of course, be expected only in arterial blood. The H_2CO_3 of venous blood is increased by absorption of CO_2 from the tissues. Consequently venous blood is less alkaline than arterial, and the difference must vary according to the activity with which the tissues perfused are producing carbon dioxide. As was shown by Zuntz (1868), the influx of CO_2 raises not only the H_2CO_3 , but by reactions such as $\text{Na}_2\text{HPO}_4 + \text{H}_2\text{CO}_3 \rightleftharpoons \text{NaH}_2\text{PO}_4 + \text{NaHCO}_3$, also raises the NaHCO_3 . Consequently the *arterial* blood bicarbonate must be accepted as the ideal measure of the alkaline reserve. In resting dogs, however, and therefore, it seems justifiable to conclude, in man, the differences between venous and arterial blood are small and fairly constant, the following being fair examples: arterial pH = 7.44, venous 7.41; arterial $\text{NaHCO}_3 + \text{H}_2\text{CO}_3 = 50$ cc. of CO_2 per 100 cc. of blood, venous = 55 cc. The differences are such that analyses of normal venous blood drawn during rest and without stasis may be regarded as but slightly inferior in accuracy and significance to those of arterial blood.

The sense in which we have used the word "*acidosis*" is not that given it by its originator, Naunyn (1906), who used the term to denote the abnormal metabolic condition in which hydroxybutyric acid is formed. The departure from this use in the literature has been a matter of evolution. Apparently because the word "*acidosis*" is suggestive of acids in general, rather than hydroxybutyric in particular, when other types of acid intoxication were discovered they also were designated as acidoses. In this broader sense the term has in recent years been used in most of the important scientific papers in the field (for example, Henderson, 1909; Palmer and Henderson, a series of papers; Barcroft, 1914; Sellards, 1914; Peabody, 1914; Howland and Marriott, 1916; Hasselbalch, 1916) "*acidosis*"

being employed to indicate the effect of acids of any type in altering the acid-base balance of the organism. We have followed these authors, rather than those who maintain the original hydroxybutyric acid definition of Naunyn. Despite the value of Naunyn's great work, it appears probable that the confused ideas of acidosis and acid intoxication that have been general have been to a considerable degree due to his definition of the term, which does not differentiate ketone production, unaccompanied by significant effect on the acid-base balance of the body, from the condition in which the acids produced do lower or abolish the reserve of alkali.

The formation of acetone bodies has a significance of its own quite apart from the secondary effect which may or may not follow on the alkaline reserve of the body. It indicates that fatty acids, derived either from fats or from amino-acids, are being incompletely oxidized. The products, β -hydroxybutyric and acetoacetic acids, may or may not be so produced and eliminated that they lower the internal alkaline reserve. We have observed an excretion of 20 gm. of acetone bodies, calculated as hydroxybutyric, per liter of urine without an abnormally low plasma bicarbonate. It is desirable that the condition in which these substances are produced be designated by a name indicating the specific nature of the metabolic abnormality and not confusing it with the general question of the acid-base balance. Rowntree proposes that the excretion of acetone bodies be indicated simply as "ketonuria," while Allen (1917) suggests for the metabolic condition which gives rise to them the equally concise and specific name of "ketosis."

II. Other Methods for Detection of Acidosis Considered as Means for the Approximate Measurement of the Arterial Blood Bicarbonate.

Most methods which have in the past demonstrated some degree of quantitative accuracy in indicating the clinical severity of acidosis are seen when analyzed to constitute approximate determinations, either direct or indirect, of the blood bicarbonate. The following are important examples.

a. Determinations Directly in the Blood.

1. *Titration of Blood.*—Titration of the blood plasma or of the filtrate obtained after precipitating the proteins with a neutral reagent is one of the oldest methods used in the study of acidosis. The source of error lies in the fact that at the high C_{H} of the end-points usually employed the titrations measure, in addition to the bicarbonate, also an acid binding power of such buffers as the phosphates, and particularly the proteins, quite out of proportion to the amounts of acid which these substances bind within the C_{H} limits that occur in the blood during life. Nevertheless, the bicarbonate seems to be the chief cause of variations in the titration figures, and results obtained by this method have consequently been of definite value in developing a knowledge of the changes that constitute acidosis (Jaksch, 1888; Magnus-Levy, 1899; Cullen, Paper III of this series).

2. *Determination of the Carbon Dioxide Content of Venous Blood.*—The use of the carbon dioxide content (CO_2 from H_2CO_3 and NaHCO_3) as a measure of the blood alkali dates back to Walter (1877), who, working in Schmiedeberg's laboratory, showed that the venous carbon dioxide of rabbits could be reduced to one-tenth its normal height by injection of acids. The significance of the determination does not differ essentially from that of the bicarbonate of the venous plasma, determined as described in this paper. In so far as the results indicate the bicarbonate of arterial blood, which must be considered as the true or compensated blood bicarbonate, the source of error lies in the fact that the blood in passing through the capillaries into the veins takes up an amount of carbonic acid which is variable with the rate of oxidation in the tissues and of blood flow through them. As a matter of experience, however, when the blood is drawn from a large vein without stasis, the difference between the venous blood and arterial appears to be sufficiently constant so that the figures for venous total carbon dioxide run approximately parallel to those for the arterial bicarbonate. The failure of Walter's method for detecting acidosis by determination of the venous CO_2 to attain general clinical use even in hospitals must be attributed chiefly to the lack of a sufficiently simple technique for the determination.

3. *Determination of the Carbon Dioxide Capacity of Venous Blood.*—In order to restore the venous blood to the condition of arterial and thus avoid the possibility of the error outlined in the above paragraph, Christiansen, Douglas, and Haldane (1914) saturated the venous blood with air containing carbon dioxide under the tension existing in normal arterial blood. As illustrated by Experiment IX, the fall caused by acidosis in the carbon dioxide capacity of venous blood is proportional to, and therefore a measure of, the fall in arterial bicarbonate.

In choosing the routine method described in this paper for measuring the alkaline reserve we have given preference to the CO_2 capacity of the *plasma*, rather than either the CO_2 content or the CO_2 capacity of venous whole blood, for practical reasons stated in the discussion of Experiment IX.

4. *Determination of the Reduced Hydrogen Ion Concentration of the Blood.*—Hasselbalch (1916, a) saturated blood with carbon dioxide at 37° under 40 mm. tension and determined under these conditions the C_H , which he calls the “reduced hydrogen ion concentration.” The H_2CO_3 concentration being fixed by the constancy of the CO_2 tension and temperature, the hydrogen ion concentration determined must vary inversely as the NaHCO_3 , since $C_H = K \frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}$. Hasselbalch’s “reduced hydrogen ion concentration” is therefore a measure of the blood bicarbonate.

5. *Determination of the Oxygen Affinity of Hemoglobin under Standard CO_2 Tension.*—Barcroft and Peters (Barcroft, 1914, p. 316) found that under changing CO_2 tension the proportion of oxygen bound by hemoglobin depended on the hydrogen ion concentration.

The value of the oxygen affinity constant, K , in the equation $\frac{y}{100} =$

$\frac{Kx^n}{1 + Kx^n}$ (y = percentage saturation of hemoglobin with oxygen, x = oxygen pressure) varies inversely as C_H . The results of Barcroft and Peters have been confirmed by Hasselbalch (1916, b). Since under a given CO_2 tension the C_H varies inversely as the NaHCO_3 , it is evident that the oxygen affinity under a given CO_2 tension is also an indirect measure of the bicarbonate; the NaHCO_3 fixes the C_H , and through it the oxygen affinity

b. Determinations on the Alveolar Air.

1. *Arterial Carbon Dioxide Tension (Haldane Method).*—The alveolar air, as shown by A. and M. Krogh (1910) is in equilibrium in respect to its carbon dioxide content with the arterial blood. Consequently, in accordance with the law of gas solubility, the concentration of carbon dioxide in the alveolar air is directly proportional to that of free carbonic acid in the blood. And the latter has been shown (p. 321), with normal respiratory control, to be kept proportional to the bicarbonate concentration. Consequently the carbon dioxide concentration of the alveolar air is, through the intermediary parallelism of the blood H_2CO_3 , kept proportional to arterial NaHCO_3 . All these concentrations go up and down together, the blood bicarbonate fixing the level of the carbonic acid, and the latter that of the alveolar carbon dioxide. Consequently in normal individuals the Haldane determination of the carbon dioxide content of air expired without previous holding of breath (Haldane and Priestley, 1905) indicates approximately the bicarbonate concentration of the arterial blood. Under pathological conditions, or under the influence of drugs, of decreased atmospheric oxygen tension, or of anxiety or excitement, the sensitiveness of the respiratory control may vary (Hasselbalch, 1912; Michaelis, 1914, p. 97; Higgins, 1915; Straub, 1915; Peters, 1917; Stillman, Van Slyke, Cullen, and Fitz, 1917), so that the alveolar carbon dioxide is not under all conditions even an approximate measure of the bicarbonate reserve. Higgins (1914) found that even changing the position of the body from standing to lying could alter the alveolar CO_2 tension to the extent of 6 mm. Sonne (1915) has shown that a mechanical error may be added to those caused by changes in the nervous control. The air collected at the end of an expiration may fail to represent the average alveolar air, instances in a normal subject being observed in which its CO_2 content was as much as 1.4 volume per cent (corresponding to 10.6 mm. tension, or one-fourth the total normal value) lower than the CO_2 content of samples of air taken near the middle of the expiration. Apparently the completeness of the gas exchange varies in different parts of the lungs. All sources of error together, however, even in pathological conditions, are in most of the cases encountered within

such limits that the clinical utility of carbon dioxide determinations in the alveolar air as a measure of the alkaline reserve of the blood is thoroughly established (Beddard, Pembrey, and Spriggs, 1915; Straub, 1915); although the fact that so many factors besides the alkaline reserve of the blood can affect the alveolar carbon dioxide tension certainly makes the latter far from an ideal measure of the former.

2. *Venous Carbon Dioxide (Plesch Method).*—The Plesch method (Plesch, 1909; Porges and Leimdörfer, 1915) differs from the Haldane in that the air analyzed, instead of being taken at the end of a single quick expiration, is breathed in and out of a rubber bag by the subject for 30 or 40 seconds. Consequently the carbon dioxide tension approaches more nearly that of the venous than of the arterial blood, the Plesch results being as a rule 4 to 6 mm. higher in carbon dioxide tension than the Haldane results. Since the venous carbon dioxide tension runs fairly parallel with the arterial, however, the Plesch results may be taken as indirect measures of the arterial bicarbonate, subject to the same errors as the Haldane results, and so to say, one degree less direct than the Haldane. An advantage of the Plesch technique is that it requires less cooperation on the part of the subject than the Haldane procedure, and has consequently been employed even with infants (Howland and Marriott, 1916).

c. *Determinations in the Urine.*

1. *Determination of the Acid Excretion.*—Since Magnus-Levy's famous paper (1899) showed the significance of β -hydroxybutyric acid as the cause of the acid intoxication in diabetic coma, it has been a matter of common observation that symptoms of acid intoxication in diabetes are usually accompanied by the excretion of large amounts of β -hydroxybutyric acid, along with lesser amounts of acetoacetic, partly as ammonium salts, but also partly as free acids. That in diabetes the excretion of free acid plus ammonia by the kidneys bears a *quantitative* relationship to the blood bicarbonate concentration is demonstrated in the accompanying paper by Fitz and Van Slyke.

2. *Alkali Retention.*—The extremely practical *alkali retention* test devised independently by Palmer and Henderson (1913) and by Sellards (1914) appears also to be an indirect measure of the bicar-

bonate content of the body fluids, as represented by the plasma. Work by Palmer, which will shortly be published in this Journal, indicates that when the plasma bicarbonate (determined as described in this paper) has reached what may be called the critical level, near the upper extreme of the normal range, urine more alkaline than blood is excreted. The amount of bicarbonate which must be taken into the organism in order to turn the urine alkaline is approximately the amount necessary to raise the bicarbonate concentration of all the body fluids to this level, if the fluids are estimated at 0.7 of the body weight and assumed to equal the plasma in bicarbonate content. The amount of alkali necessary to administer in the retention test appears consequently to be proportional to the margin by which the plasma bicarbonate falls below the critical level at the time of administration, and therefore constitutes an indirect measure of the plasma bicarbonate.

III. The Influence of Free Carbonic Acid Concentration in the Blood on the Plasma Bicarbonate.

The plasma bicarbonate concentration is influenced by the free carbonic acid concentration, both of the whole blood at the time the plasma is separated from the cells, and of the plasma itself at the time the determination is made. The influence is exerted respectively through affecting the distribution of acids and bases between plasma and corpuscles, and through affecting reactions within the plasma itself. Both modes of influence must be considered in connection with any method for determining the concentration of plasma bicarbonate in venous blood, and we shall therefore discuss them from the standpoint of their effects on such determinations.

a. Influence through Effect on Equilibria within the Plasma.—To a minor extent the bicarbonate of the plasma can be affected by the equilibrium between normal carbonate, bicarbonate, and carbonic acid: $2\text{NaHCO}_3 \rightleftharpoons \text{Na}_2\text{CO}_3 + \text{H}_2\text{CO}_3$. As shown by Bohr, however, the conditions of this equilibrium are such that, with the concentrations of free carbonic acid existing in the plasma during life, the proportion of Na_2CO_3 is from a quantitative standpoint negligible, practically all the alkali not bound by other acid than carbonic being in the form of bicarbonate.

In a 0.155 per cent sodium carbonate solution (about the average carbonate concentration of plasma) at 38°, and with the physiologically normal carbon dioxide tension of 45 mm., Bohr calculated that 99.5 per cent of the sodium carbonate was in the form of bicarbonate, and confirmed the calculation experimentally within the limit of analytical error. Even at 12 mm. CO₂ tension, which is seldom if ever observed in life except in premortal coma, 98 per cent was in the form of bicarbonate. Consequently one can, for quantitative purposes, regard the bicarbonate of the plasma as synonymous with its entire reserve of alkali in excess of that neutralized by acids other than carbonic.

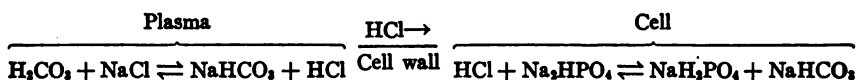
There are other equilibria than that between carbonate and bicarbonate, however, which are more sensitive to changes in H₂CO₃ concentration. If carbon dioxide escapes from a sample of plasma, the latter loses not only free carbonic acid CO₂, but also part of the CO₂ normally combined as bicarbonate, which undergoes partial decomposition by such reversible reactions as $\text{NaHCO}_3 + \text{protein} \rightleftharpoons \text{H}_2\text{CO}_3 + \text{Na proteinate}$. This reaction of the proteins appears in fact to be the one chiefly responsible for the variation in plasma bicarbonate caused by varying free carbonic acid (see Experiments II and III). (While in the cells the reaction $\text{NaHCO}_3 + \text{NaH}_2\text{PO}_4 \rightleftharpoons \text{Na}_2\text{HPO}_4 + \text{H}_2\text{CO}_3$, studied by Henderson (1906), is important, in the plasma the phosphate concentration is too small to affect appreciably the bicarbonate (Greenwald, 1915).) If the H₂CO₃ falls greatly below normal even the reaction $2\text{NaHCO}_3 \rightleftharpoons \text{H}_2\text{CO}_3 + \text{Na}_2\text{CO}_3$ becomes appreciable. In each of these reactions a decrease in the free carbonic acid results in a shift of the equilibrium from left to right, and consequently in a decrease of the bicarbonate. Thus Jaquet found that at 42.7 mm. CO₂ tension the bicarbonate CO₂ of a normal plasma sample was 63.7 volume per cent, while at 17 mm. it was 58.5 per cent. The difference, though not great, is considerable, and becomes accentuated as the carbon dioxide tension is reduced still lower. Consequently in a solution such as the plasma the term bicarbonate content has a quantitatively definite meaning *only for a definite concentration of free carbonic acid*.

One has the choice of two alternatives. One may vary the free carbonic acid in proportion to the bicarbonate, maintaining the 1:20 ratio, and thus determining the genuine "compensated" bicarbonate which exists in the arterial blood. Or one may make all determinations at a fixed and definite carbonic acid concentration.

The first plan has the theoretical advantage of duplicating natural conditions, but is impracticable for a routine method, as it would necessitate the use of a different carbon dioxide mixture in saturating every plasma. We have therefore, in the method described in this paper, adopted the plan of saturating all plasmas with carbon dioxide under normal alveolar tension. This has the theoretical disadvantage that in extreme acidosis the bicarbonate determined is not quite so low as that actually existing in the arterial blood. The fall below normal, however, is parallel to that of the arterial bicarbonate (see Experiment IX) and, as a matter of fact, the absolute difference between results by the two methods is not great. For example, the plasma of a diabetic patient with marked acidosis showed, when saturated with CO_2 at the reduced alveolar CO_2 tension of the patient, a bicarbonate yielding 23 cc. of CO_2 per 100 cc. of plasma, while the figure obtained after saturating with CO_2 under normal tension was 26 cc. We believe that under the conditions of constant CO_2 tension chosen the results are no less definite in their significance than they would be if we attempted to approximate the varying CO_2 tension existing in arterial blood.

b. Influence of Carbonic Acid on the Plasma Bicarbonate through Effect on the Transfer of Electrolytes between Plasma and Cells.—Gürber (1895) noticed that as the result of saturating the blood with carbon dioxide *in vitro* the titratable alkali of the plasma, which includes the bicarbonate, was increased. This phenomenon could be explained by assuming either that alkali diffuses from cells into plasma to meet the increased carbonic acid there, or that acids other than carbonic are, so to say, forced by the carbonic from the plasma into the cells, leaving in the form of bicarbonates the alkali with which they had been combined. Gürber claimed that no potassium or sodium at all passed from corpuscles into plasma when blood was saturated with carbon dioxide, that the entire change was due to passage of HCl from the plasma into the cells, the amount which passed being equivalent to the gain in titratable alkali in the plasma. As shown by Experiment X, however, the amount of HCl which disappears from plasma of blood saturated with pure CO_2 is equivalent only to about one-third the increase in bicarbonate. Hamburger (1916) has recently shown, furthermore, there is some transfer of K

and Na between plasma and cells. Consequently Gürber's belief that transfer of HCl alone was responsible for the alkali shift caused by saturating blood with CO₂ does not hold. That the alterations which occur within physiological limits of CO₂ tensions are chiefly due to transfer of HCl appears probable, however (see Experiment X). The reaction in the plasma, the consequent HCl transfer, and reaction of the transferred acid with phosphates inside the cell may be formulated as follows:



i.e., although the buffer salts may not readily pass out to neutralize acid in the plasma, the acid does pass in to meet the salts within the cells, so that the same effect is obtained in maintaining plasma neutrality. In fact Hamburger, to whose thorough researches we owe most of our knowledge in this field, has shown that it is highly probable that the corpuscles only typify the body cells in general, and that the transfer of acid to and from the latter is of such a nature that the plasma has practically all the buffer salts of the body at its disposal in maintaining its neutrality, despite the fact that it itself is not particularly rich in such salts. In confirmation of this view see Section 2 of the discussion of the results of Experiment IX.

The magnitude of the effect on plasma bicarbonate which can be caused by such loss of carbon dioxide as may occur when blood is drawn into an open receptacle may be seen by reference to Experiment VII, while the extreme effects obtainable, on the one hand by removing CO₂ as completely as possible, on the other by saturating the blood with pure CO₂ gas, are shown by Experiment X.

It is evident that in fixing conditions for the determination of the plasma bicarbonate as a measure of acidosis the concentration of free carbonic acid not only in the plasma at the time of the determination, *but also in the whole blood at the time the corpuscles are separated* from the plasma, must be considered.

The ideal determination would be made on arterial blood drawn without loss of CO₂, for only in the arterial blood is the constancy of the $\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}$ ratio exactly maintained. The use of venous blood

from human subjects being necessary, however, the nearest approximation to arterial conditions would be obtained by saturating the blood at body temperature with CO_2 at such a tension that the normal value of the $\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}$ ratio in the blood would be maintained. This can be approximated by saturating the blood at body temperature with the alveolar air of the subject. In clinical routine, however, such a procedure adds to the technique a complicating factor which our experience indicates is unnecessary. An alternative would be to determine the "carbon dioxide capacity" of the blood after saturating the whole blood with carbon dioxide at the average normal alveolar tension. This method and the practical drawback to its routine application are discussed on pp. 326 and 336. It proved in fact to be entirely practicable to centrifuge the blood as it was obtained directly from the arm vein.

Method for Determining the Plasma Bicarbonate under Constant Dioxide Tension.

I. Drawing Blood Sample.—For at least an hour before the blood is drawn the subject should avoid vigorous muscular exertion, as this, presumably because of the lactic acid formed, lowers the bicarbonate of the blood (Christiansen, Douglas, and Haldane, 1914; Morawitz and Walker, 1914). The blood is drawn from the arm vein directly into a centrifuge tube containing enough powdered potassium oxalate to make about 0.5 per cent the weight of the blood drawn. In order to avoid accumulation of carbon dioxide and consequent effect on the electrolyte transfer between plasma and cells, it is desirable to avoid stasis, or when stasis is necessary, to release the ligature as soon as the vein is entered and allow a few seconds for the stagnant blood to pass. This is particularly important when the second procedure for collecting blood described below is used, for in this case no opportunity is given for excess of free CO_2 to escape.

For drawing the blood we have used two methods. For clinical purposes the McRae needle⁴ was chiefly employed (first method).

⁴ The McRae blood needle may be obtained from the Kny-Scheerer or the Tiemann Company of New York. The principle is similar to that of the Hallion and Bauer needle, described in *Compt. rend. Soc. biol.*, 1912, lxxii, 232.

With this, the blood enters the collecting tube in a fine stream and falls through a height of several centimeters before it reaches the bottom. During this fall there is opportunity for the escape of carbon dioxide and for absorption of oxygen. The carbon dioxide loss, by its effect on the transfer of HCl between plasma and corpuscles, discussed in the preceding pages, measurably lowers the bicarbonate content of the plasma. As a matter of experience, however, the gas exchange which occurs in the interval of about 0.01 second during which the blood is falling is only enough to bring the carbonic acid content of the venous blood to approximately that of arterial (see Experiment VII). The tube is turned on its side and back to vertical position once or twice after the sample has been drawn, in order to mix the oxalate. *The blood is subjected to no other agitation* which might accelerate loss of carbon dioxide, and is centrifuged in the same tube within a few minutes after it has been drawn (for effect of standing see Table X). The results of Stillman, Van Slyke, Cullen, and Fitz (Paper VI of this series), who compared the CO_2 capacity of plasma drawn by this technique with the CO_2 capacity of the whole blood, indicate that the McRae tube can be used in routine clinical work, provided the above precautions are observed, without fear of error.

The other method is to avoid all loss of carbon dioxide and obtain strictly venous blood. For this purpose ordinary care in the use of a syringe is sufficient, the blood being drawn without suction, and free air space in the syringe being avoided. A satisfactory substitute for the syringe is shown in Fig. 1. The blood is collected and centrifuged under paraffin oil. The slight amount of agitation necessary in order to assure mixture of the oxalate is accomplished by stirring with the inlet tube, rather than by inverting or shaking. The paraffin oil, like most organic liquids, dissolves carbon dioxide in greater amounts than does water, and its action in preventing loss of carbon dioxide from the blood is due to prevention of free diffusion from the surface of the water rather than to the formation of a layer impermeable to gas. Consequently the tube is subjected to a minimum of agitation after the blood is in it. When this precaution is taken, the results are the same as those from blood drawn with a syringe (see Experiment VII, *d*).

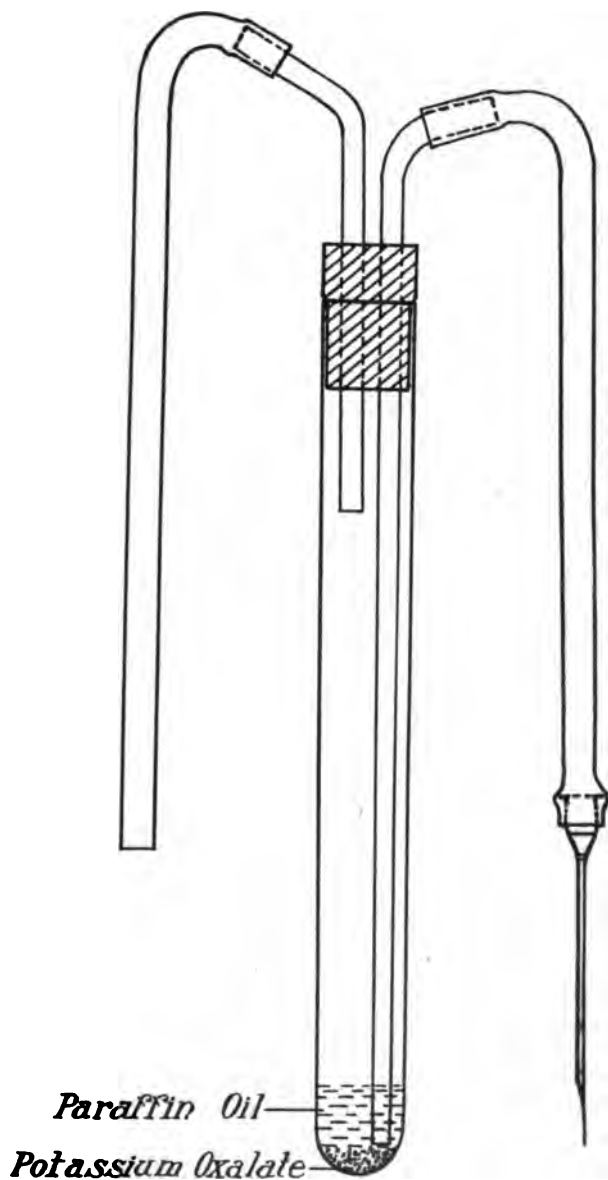


FIG. 1. Centrifuge tube arranged for collecting blood under paraffin oil without loss of carbon dioxide.

When blood is drawn with momentary exposure to air (McRae needle) and analyzed in the manner described in this paper, the plasma being resaturated with air containing 5.5 per cent of CO_2 , results of our own and the analyses of thirty normal bloods by Gettler and Baker (1916) show that the bicarbonate CO_2 yielded by 100 cc. of normal plasma varies from 53 to 75 cc., reduced to 0° , 760 mm. A great majority of plasmas show figures between 60 and 70 cc. Occasionally an alkaline diet may force the figure to the upper limit of 80 cc., but we have not yet seen it below 53 in any normal person.

With the second method (venous blood drawn under oil without loss of CO_2) the results of Austin and Jonas (1917) agree with the relatively small number of determinations on human subjects which

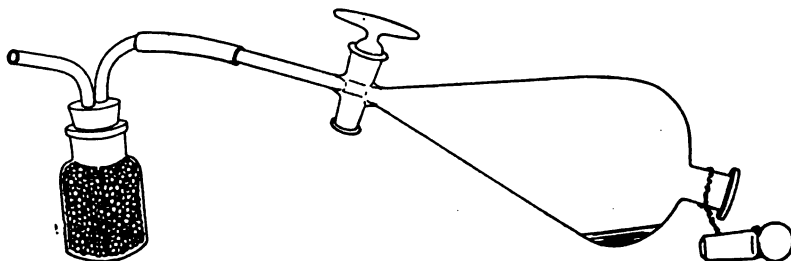


FIG. 2. Separatory funnel containing plasma and arranged for filling with alveolar air.

we have made in placing the minimum normal figure at 60 instead of 53 cc. of CO_2 per 100 cc. of plasma. With either procedure, but especially with this one, where there is no opportunity for the escape of excess CO_2 , the remarks in the first paragraph of this section concerning the avoidance of stagnation are pertinent.

If the difference in the level of the minimum normal values is kept in mind, it appears that the two methods of blood drawing may be used interchangeably (see, for example, Experiment VII, *e*). In this hospital we have used chiefly the McRae needle, but Austin and Jonas, to whom we have communicated both methods and who have tried both, prefer the oil tube.

II. Separation and Storage of Plasma for Analysis.—While results of similar significance are obtained by analysis of either whole blood

or plasma, we prefer the plasma for routine determinations for the following reasons. It can be measured and handled with greater convenience than whole blood. The oxygen bound by the hemoglobin does not complicate the determination when plasma is used. And, perhaps the most important reason, plasma can be kept for a long time without alteration in its carbon dioxide binding capacity, while whole or defibrinated blood begins to show a decrease in its alkaline reserve soon after it has been drawn. This apparent formation of acid in blood in which the corpuscles remain was discovered by Christiansen, Douglas, and Haldane, who found that at 37°, even within an hour after blood had been drawn and defibrinated, an appreciable fall in the carbon dioxide capacity occurs, so that they were able to obtain comparable results only when a constant interval, as short as possible, was allowed to elapse between the drawing of the samples and the determination of their carbon dioxide capacities. The change observed can be most readily explained as due to the formation of acids in the blood cells. The change certainly does not occur in their absence, for we find that sterile plasma, if kept cold and in tubes that have been paraffined in order to avoid solution of a salt from the glass, can be preserved for over a week without alteration in its carbon dioxide capacity. It may be well to state here, however, that plasma in ordinary glass can be kept for only a few hours, as sufficient alkali dissolves from the glass in longer intervals to increase measurably the carbon dioxide capacity.

In case it is necessary to separate the plasma by gravity, the sedimentation is allowed to occur in a closed tube which is completely filled with blood, so that no carbon dioxide can escape, and the plasma is drawn off in as short a time as possible.

Since during rest and normal circulation the carbon dioxide content of the venous blood is only a few per cent higher than that of the arterial, and the difference does not vary greatly, the plasma obtained without stasis might be analyzed at once and without further preparation. As a matter of routine, however, we have found it desirable to saturate the plasma with carbon dioxide at a definite tension, as described below, immediately before analysis, and thus avoid the possibility of error caused by loss of carbon dioxide while the sample is awaiting analysis.

III. Saturation of Plasma with Air Containing Carbon Dioxide under Normal Alveolar Tension.—For the saturation we have found the most convenient vessels to be ordinary separatory funnels capable of holding about 100 times the volume of the plasma that is to be saturated. The plasma is placed in the funnel, the latter is turned on its side, and the air within is displaced by either alveolar air from the lungs of the operator or with 5.5 per cent CO_2 -air mixture from a tank. In either case the gas mixture must be passed over glass beads before it enters the funnel (see Fig. 1). Otherwise, when air from the lungs is used the plasma is appreciably diluted with the moisture which condenses from the breath on the inner walls of the funnel. By passage over a large surface of either wet or dry glass beads at room temperature the expired air is cooled, and the excess moisture in it is condensed, so that not enough is carried into the funnel to cause an appreciable error. When, on the contrary, a dry CO_2 -air mixture from a tank is used, it causes an appreciable evaporation from the surface of the plasma, with consequent increase in its concentration and in the carbon dioxide capacity. This also is obviated if the gas mixture is passed over wet beads, so that it approaches saturation with water vapor.

For obtaining an artificial mixture of air containing 5.5 per cent of CO_2 we have used an ordinary metallic gas tank capable of standing 20 atmospheres pressure and provided with an accurate pressure gage. Carbon dioxide was run in from another tank until the desired pressure was indicated. Then air was run in until the total pressure of air plus CO_2 was 18.2 times that of the CO_2 (taking into account that the tank contains one atmosphere more than the gage registers). The tank was then laid on its side for a half hour to give the gases an opportunity to mix thoroughly, and samples were drawn for analysis before the mixture was used. The analysis had to be repeated every few days, as the CO_2 content of the gas sometimes changes unexplainably. In order to displace completely the air in the separatory funnel with the CO_2 mixture, five or more volumes were run through, the gas, after leaving the funnel, being collected in a gasometer or rubber bag so that the volume passed could be roughly estimated.

When alveolar air is used, the operator, without inspiring more deeply than normal, expires as quickly and as completely as possible through the bottle of glass beads and the separatory funnel connected as shown in Fig. 2. The stopper is inserted just before the expiration is finished, so that there is no opportunity for air to be drawn back into the funnel. With a little practice a normal person can consis-

tently fill a 300 cc. separatory funnel with air containing within a few tenths of a per cent of the desired 5.5 per cent of CO_2 . The composition is not, of course, so constant as that obtained when an analyzed gas mixture is used to fill the funnel, but as a matter of experience we have never found that the deviations caused significant error in the results. A change of 0.5 per cent in the CO_2 concentration of the air with which the plasma is shaken causes a change of only about 1 volume per cent in the plasma in the amount of CO_2 gas taken up, of which the total is normally 60 to 80 volumes per cent.

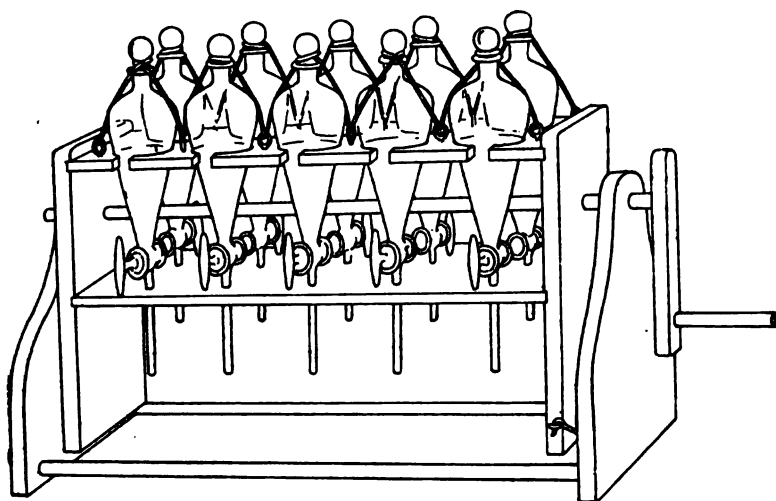


FIG. 3. Rack for holding separatory funnels during saturation of plasma. The elastic cord which holds the stoppers, as well as the entire funnels, in place, is made of spiral wire.

The following figures exemplify the effect of CO_2 concentration in the air on the amount of carbon dioxide taken up by plasma. Samples of the same plasma were shaken in atmospheres of air containing respectively 3.2, 5.5, and 9.6 per cent of carbon dioxide. The results were:

Volume per cent of CO_2 in air.	Gas obtained from 1 cc. of plasma.	Change in absorbed CO_2 due to 1 per cent change in CO_2 of air.
<i>per cent</i>	<i>cc.</i>	<i>cc.</i>
3.2	0.584	0.023
5.5	0.636	0.016
9.6	0.700	

In order to saturate the plasma the separatory funnel is turned end over end for 2 minutes, the plasma being distributed in a thin layer as completely over the surface of the funnel's interior as is possible. We have found that 2 minutes' shaking in this manner uniformly suffices for saturation, but that 1 minute is as a rule not enough. When there are several analyses to be done it is convenient to use a rotating rack such as is shown in Fig. 3. In this ten separatory funnels can be shaken at once, and the rack acts as a holder for them at other times.

As a rule, when plenty of plasma is available, we saturate 3 cc. of it in a 300 cc. separatory funnel. One then has sufficient for duplicate determinations on 1 cc. each. As it is possible even with the large apparatus to make a determination with 0.5 cc. of plasma, one can, when the supply is scanty, saturate a little more than 0.5 cc. in a 50 cc. funnel. In this case the volume of distilled water and acid used to wash the plasma into the apparatus is also halved, so that the total volume of water solution introduced is only 1.25 cc. The volume of gas observed is multiplied by 2 before it is used to calculate the volume per cent of CO_2 bound; *i.e.*, a considerable error would be caused if the CO_2 capacity were first calculated from the observed reading, and the result multiplied by 2 *after* the calculation.

When the micro-apparatus for carbon dioxide determinations described in the next paper is used, one-fifth the above amounts of plasma suffices.

IV. Determination of Carbon Dioxide Content of the Saturated Plasma.—After saturation is completed the funnel is placed upright and allowed to stand a few minutes until the fluid has drained from the walls and gathered in the contracted space at the bottom of the funnel. A sample of 1 or 0.5 cc. for the large apparatus, or 0.2 cc. for the micro-apparatus, is drawn with a calibrated pipette and used for the determination of the carbon dioxide content, which is performed as described in the next paper.

When the plasma is being delivered from the pipette into the cup of the apparatus, the tip of the pipette is held below the surface of the liquid in the cup. If the plasma were allowed to run through the air in a fine stream loss of carbon dioxide would result.

It is convenient to use a small drop (0.02 cc.) of octyl alcohol to prevent foaming of the plasma and emulsification of the mercury.

The gas volume is measured after a single extraction, and the result is calculated, by means of the table on p. 344 into terms of volume per cent of carbon dioxide gas, measured at 760 mm., 0°, which is bound as bicarbonate by the plasma.

The carbon dioxide combining capacity of plasma varies appreciably with the temperature, so that human plasma at 20° binds as bicarbonate approximately 106 per cent as much carbonic acid as at 37°. The extent of the temperature effect is demonstrated and its nature discussed in connection with Experiment IV. After determining the temperature coefficients of a number of plasmas we have been able to introduce the average coefficient into the calculation, so that both saturation and analysis can be performed at room temperature without significantly affecting the constancy or reproducibility of the results.

V. Calculation of Results.—When from plasma, saturated as above described with alveolar air, gases are extracted for analysis one obtains not only the CO₂ bound as bicarbonate and set free by acidification, but also the CO₂ and air physically dissolved by the plasma and water. The gases thus dissolved are, of course, independent of the alkaline reserve, and are subtracted from the total in order that the carbon dioxide bound as bicarbonate may be estimated. The exact amount to be subtracted, which is about 0.10 cc. when 1 cc. of plasma is analyzed, but varies slightly with the room temperature, may be determined by blank analyses, or calculated from the known solubility coefficients of the gases.

a. Determination of Correction for Dissolved Gases by Blank Analysis.—A few cc. of acidulated water are saturated with alveolar air or 5.5 per cent CO₂, as described above, and 1 cc. is analyzed with the same technique used for plasma. The total amount of gas obtained is the “dissolved gas” correction. When subtracted from the volume of gas obtained in a plasma analysis made under similar conditions of temperature and pressure, the difference represents the CO₂ chemically bound as bicarbonate in the plasma. This value is multiplied by the factor given in column C, Table I of the next paper, which both reduces the gas to standard conditions (0°, 760 mm.) and cor-

rects for the 4 or 5 per cent of the total CO_2 not removed from the water by the single extraction.

b. Formula Including Corrections for Temperature and Dissolved Gases. Table for Calculation of Results.—In order to calculate the carbon dioxide chemically bound (as bicarbonate) the basic equation of Paper II must be altered by introducing a term which deducts from the total carbon dioxide content of the plasma the amount dissolved as free carbonic acid. This amount in cc. of carbon dioxide gas per cc. of plasma is equal to $p\alpha_{\text{CO}_2}$ when the plasma is in equilibrium with air containing p proportion of carbon dioxide by volume, α_{CO_2} being the solubility coefficient of carbon dioxide in water. Introducing this subtraction into our basic equation, the latter becomes

Cc. of CO_2 chemically bound by 1 cc. of plasma.	Factor for re- ducing gas volume to standard con- ditions, 0° , 760 mm.	Observed gas volume in cc.	Cc. of air carried into apparatus dissolved in 2.5 cc. of solution.
(1) $x =$	f	{ V	$-(2.5 - p) \alpha_{\text{air}}$

Cc. of CO_2 kept in solution after first extraction.	Cc. of CO_2 dissolved in 1 cc. plasma as free carbonic acid.
$+ 0.053 (V - [2.5 - p] \alpha_{\text{air}}) \alpha_{\text{CO}_2}$	$- 0.975 p \alpha_{\text{CO}_2}$

The term $(2.5 - p) \alpha_{\text{air}}$ is derived as follows. The volume of air dissolved in 1 cc. of plasma shaken with air containing p proportion of carbon dioxide is $(1 - p) \alpha_{\text{air}}$. The volume held in solution under atmospheric pressure by the 1.5 cc. of water and dilute acid used in washing the plasma into the apparatus is $1.5\alpha_{\text{air}}$. Hence the total correction for the air in the gas volume observed is $(1 - p) \alpha_{\text{air}} + 1.5 \alpha_{\text{air}}$, or $(2.5 - p) \alpha_{\text{air}}$. When p is only 0.055, as is the case when determining the carbon dioxide capacity of plasma in the manner described in this paper, its effect on this term is negligible; but if air containing proportions of carbon dioxide much higher than the physiological 5.5 per cent was utilized in saturating the plasma the effect of p would become measurable. This was the case in some experiments to be reported later, and the value of p is introduced into the equation so that it can be used in such cases.

The derivation of the other terms is self-evident. The last term has the coefficient 0.975 because Bohr has shown that the dissolved substances in plasma reduce the solubility of gases in it to 97.5 per cent of their solubilities in pure water. As in the case of p , this factor, 0.975, exerts an appreciable influence on the results calculated only when the plasma is saturated with gas containing a

much higher percentage of carbon dioxide than the 5.5 per cent used in routine determinations of carbon dioxide capacities.

For routine determinations the equation is reduced to a working basis by substituting 0.055 for p , and by introducing the temperature coefficients for the various constants, in the manner employed in the derivation of Equation 4 of Paper II. We then have

$$(2) \quad x = \frac{B}{760} (107.3 - 0.586 t) (V - 0.136 + 0.002 t)$$

t being the temperature centigrade.

As will be shortly shown, however, the carbon dioxide combining capacity of plasma decreases by an average of 0.36 per cent for each degree rise in the temperature at which the plasma is saturated with the CO_2 -air mixture. In order to have results obtained at different room temperatures accurately comparable, therefore, we have introduced this additional temperature coefficient into the calculation in such a manner that the results calculated indicate the amount of carbon dioxide the plasma would bind if it were saturated at 20° . Introducing the temperature coefficient 0.0036 in this manner, we have

$$(3) \quad x = \frac{B}{760} \frac{107.3 - 0.586 t}{1 + 0.0036 (t - 20)} (V - 0.136 + 0.002 t)$$

The values of

$$\frac{107.3 - 0.586 t}{1 + 0.0036 (t - 20)}$$

may be accurately expressed between 15° and 30° by the term $100.8 - 0.27 t$. Hence the equation becomes

$$(4) \quad x = \frac{B}{760} (100.8 - 0.27 t) (V - 0.136 + 0.002 t)$$

x expressing the cc. of CO_2 reduced to 0° , 760 mm., which 1 cc. of plasma will bind as bicarbonate when in equilibrium at 20° with air containing 5.5 per cent by volume of carbon dioxide.

TABLE 1.

Table for Calculation of Carbon Dioxide Combining Power of Plasma.

Observed vol. gas $\times \frac{B}{760}$	Cc. of CO ₂ reduced to 0°, 760 mm., bound as bicarbonate by 100 cc. of plasma.				Observed vol. gas $\times \frac{B}{760}$	Cc. of CO ₂ reduced to 0°, 760 mm., bound as bicarbonate by 100 cc. of plasma.			
	15°	20°	25°	30°		15°	20°	25°	30°
0.20	9.1	9.9	10.7	11.8	0.60	47.7	48.1	48.5	48.6
1	10.1	10.9	11.7	12.6	1	48.7	49.0	49.4	49.5
2	11.0	11.8	12.6	13.5	2	49.7	50.0	50.4	50.4
3	12.0	12.8	13.6	14.3	3	50.7	51.0	51.3	51.4
4	13.0	13.7	14.5	15.2	4	51.6	51.9	52.2	52.3
5	13.9	14.7	15.5	16.1	5	52.6	52.8	53.2	53.2
6	14.9	15.7	16.4	17.0	6	53.6	53.8	54.1	54.1
7	15.9	16.6	17.4	18.0	7	54.5	54.8	55.1	55.1
8	16.8	17.6	18.3	18.9	8	55.5	55.7	56.0	56.0
9	17.8	18.5	19.2	19.8	9	56.5	56.7	57.0	56.9
0.30	18.8	19.5	20.2	20.8	0.70	57.4	57.6	57.9	57.9
1	19.7	20.4	21.1	21.7	1	58.4	58.6	58.9	58.8
2	20.7	21.4	22.1	22.6	2	59.4	59.5	59.8	59.7
3	21.7	22.3	23.0	23.5	3	60.3	60.5	60.7	60.6
4	22.6	23.3	24.0	24.5	4	61.3	61.4	61.7	61.6
5	23.6	24.2	24.9	25.4	5	62.3	62.4	62.6	62.5
6	24.6	25.2	25.8	26.3	6	63.2	63.3	63.6	63.4
7	25.5	26.2	26.8	27.3	7	64.2	64.3	64.5	64.3
8	26.5	27.1	27.7	28.2	8	65.2	65.3	65.5	65.3
9	27.5	28.1	28.7	29.1	9	66.1	66.2	66.4	66.2
0.40	28.4	29.0	29.6	30.0	0.80	67.1	67.2	67.3	67.1
1	29.4	30.0	30.5	31.0	1	68.1	68.1	68.3	68.0
2	30.3	30.9	31.5	31.9	2	69.0	69.1	69.2	69.0
3	31.3	31.9	32.4	32.8	3	70.0	70.0	70.2	69.9
4	32.3	32.8	33.4	33.8	4	71.0	71.0	71.1	70.8
5	33.2	33.8	34.3	34.7	5	71.9	72.0	72.1	71.8
6	34.2	34.7	35.3	35.6	6	72.9	72.9	73.0	72.7
7	35.2	35.7	36.2	36.5	7	73.9	73.9	74.0	73.6
8	36.1	36.6	37.2	37.4	8	74.8	74.8	74.9	74.5
9	37.1	37.6	38.1	38.4	9	75.8	75.8	75.8	75.4
0.50	38.1	38.5	39.0	39.3	0.90	76.8	76.7	76.8	76.4
1	39.1	39.5	40.0	40.3	1	77.8	77.7	77.7	77.3
2	40.0	40.4	40.9	41.2	2	78.7	78.6	78.7	78.2
3	41.0	41.4	41.9	42.1	3	79.7	79.6	79.6	79.2
4	42.0	42.4	42.8	43.0	4	80.7	80.5	80.6	80.1
5	42.9	43.3	43.8	43.9	5	81.6	81.5	81.5	81.0
6	43.9	44.3	44.7	44.9	6	82.6	82.5	82.4	82.0
7	44.9	45.3	45.7	45.8	7	83.6	83.4	83.4	82.9
8	45.8	46.2	46.6	46.7	8	84.5	84.4	84.3	83.8
9	46.8	47.1	47.5	47.6	9	85.5	85.3	85.2	84.8
0.60	47.7	48.1	48.5	48.6	1.00	86.5	86.2	86.2	85.7

The temperature figures at the heads of columns represent the room temperatures at which the samples of plasma are saturated with alveolar CO_2 and analyzed. It is assumed that both operations are performed at the same temperature. The figures have been so calculated that, regardless of the room temperature at which saturation and analysis are performed, the table gives the volume (reduced to 0° , 760 mm.) of CO_2 that 100 cc. of plasma are capable of binding when saturated at 20° with CO_2 at approximately 41 mm. tension. If the figures in the table are multiplied by 0.94 they give, within 1 or 2 per cent, the CO_2 bound at 37° .

If the figures in the table are multiplied by 0.66 they give the mm. CO_2 tension of the alveolar air (Haldane method) if the relationship between alveolar carbon dioxide and plasma bicarbonate is the *average* normal. The physiological deviations from this average may be as great as 7 mm. (Paper V), the pathological much greater (Paper VI).

For convenience in the calculation the values for the ratio $\frac{\text{barometer}}{760}$ over the range usually encountered are given below.

Barometer.	$\frac{\text{Barometer.}}{760}$	Barometer.	$\frac{\text{Barometer.}}{760}$
732	0.961	756	0.995
734	0.996	758	0.997
736	0.967	760	1.000
738	0.971	762	1.003
740	0.974	764	1.006
742	0.976	766	1.008
744	0.979	768	1.011
746	0.981	770	1.013
748	0.984	772	1.016
750	0.987	774	1.018
752	0.989	776	1.021
754	0.992	778	1.024

In order to express results in mg. of CO_2 bound by 1 cc. of plasma, the factor $\frac{\text{weight in mg. of 1 cc. CO}_2}{100} = 0.01964$ may be introduced, yielding

(5) Mg. CO_2 bound chemically by 1 cc. plasma

$$= \frac{B}{760} (1.982 - 0.0053 t) (V - 0.136 + 0.002 t)$$

In order to avoid the necessity of calculation in routine work, we have computed Table I by means of Equation 4. By means of this

table the readings on the apparatus can be directly transposed into cc. of CO_2 chemically bound by 100 cc. of plasma. It will be seen that when the gas volume reading is above 0.50 cc. the various temperature effects nearly neutralize each other, so that a reading of 0.70 cc., for example, indicates almost exactly the same carbon dioxide capacity, whether saturation and analysis are performed at 15° or at 25° .

EXPERIMENTAL.

I. The Non-Effect of Potassium Oxalate on the Carbon Dioxide Capacity of Plasma.

a. The Effect of Oxalate on the Carbon Dioxide Capacity of Water.—Before oxalate could be used to prevent coagulation of blood it was necessary to demonstrate that its introduction causes no appreciable change in the carbon dioxide capacity. As shown by the following experiment, a solution of potassium oxalate dissolves under given conditions more carbon dioxide than does pure water.

Water and solutions containing respectively 1 and 10 per cent of potassium oxalate were shaken at 29° with air containing 23.0 per cent of carbon dioxide by volume. The carbon dioxide content of the solutions was determined as described in the preceding paper, with the exception that 2 cc. of each solution instead of 1 cc. were taken for analysis. The following results were obtained.

TABLE II.

Solution.	Vol. of CO_2 dissolved.	α CO_2 observed.	α CO_2 observed by Bohr and Bock.
Water.....	0.345	0.75	0.753
1 per cent oxalate.....	0.435	0.92	—
10 " "	0.480	1.04	—

It is evident that the oxalate imparts to pure water a slight alkalinity which can be measured by so delicate a means as the carbon dioxide capacity. Pure water is immensely more sensitive to the effects of solutes of slightly acid or alkaline nature, however, than are solutions containing buffers, like carbonates, phosphates, and proteins. Blood is preeminently such a solution, and in order to make experi-

ments on the effect of oxalate applicable to blood plasma, they must be performed upon solutions which imitate the buffer composition of the plasma.

b. Effect of Oxalate on the Carbon Dioxide Capacity of Phosphate Solutions.—Soerensen's solutions of pure phosphates in M/15 concentration were used. 10 cc. of KH_2PO_4 solution and 40 cc. of Na_2HPO_4 solution were mixed, the resulting solution having a pH of 7.38, approximately that of the blood. The carbon dioxide capacity of this solution was determined in exactly the manner described in the preceding pages for plasma, except that 5.0 per cent CO_2 -air mixture instead of 5.5 per cent was used. The following results were obtained.

TABLE III.

Solution used.	Vol. gas observed.	CO_2 dissolved.
	cc.	mg.
Phosphate.....	0.260	0.396
	0.265	0.405
Phosphate + 1 per cent oxalate.....	0.260	0.396
	0.262	0.400
Phosphate + 10 per cent oxalate.....	0.260	0.396
	0.262	0.400

The determinations were made at 24° , 760 mm., and the results in mg. calculated by the formula for 24° given in Table I of the succeeding article.

It is evident that oxalate, even up to 10 per cent concentration, does not affect the carbon dioxide capacity of the phosphate solution.

c. Effect of Oxalate on the Carbon Dioxide Capacity of Sodium Carbonate Solution.—A 0.1 per cent solution of sodium carbonate was used for the experiment which in all details was similar to that preceding. The carbon dioxide capacity of this solution is slightly less than that of normal plasma.

TABLE IV.

Solution used.	Vol. gas observed at 24° , 760 mm.	Total CO_2 dissolved.	CO_2 chemically bound.
	cc.	mg.	mg.
0.1 per cent Na_2CO_3	0.534	0.90	0.826
0.1 per cent Na_2CO_3 + 1 per cent oxalate.....	0.529	0.89	0.816
0.1 per cent Na_2CO_3 + 10 per cent oxalate.....	0.515	0.865	0.791

The presence of 1 per cent of oxalate had no effect on the carbon dioxide capacity of 0.1 per cent Na_2CO_3 solution outside the limit of error of the determination. 10 per cent of oxalate did not increase, but reduced the carbon dioxide capacity of the solution detectibly, an effect which may be attributed to the fact that the addition of so much solid oxalate to the carbonate solution appreciably increased its volume, so that it contained less than 1 mg. of Na_2CO_3 per cc. To a minor degree the effect is also due to the well known fact that the presence of salts reduces the solubility of gases in water, so that less CO_2 is dissolved as free H_2CO_3 than in pure water solution.

The results of both preceding experiments show that potassium oxalate in 1 per cent and even greater concentration does not affect the carbon dioxide capacities of solutions containing concentrations of phosphate or of sodium carbonate such as would bind the amounts of carbon dioxide held by the plasma. Consequently the oxalate appears to be excluded as a source of error in our determinations on plasma.

It may be noted that the amounts of CO_2 bound as carbonate, 0.826 mg. in absence of oxalate, 0.816 mg. in the presence of 1 per cent oxalate, are very near the amount, 0.830 mg., that must be bound to convert all the carbonate into NaHCO_3 . The results harmonize with those of Bohr, who determined both by calculation and by analysis that nearly 100 per cent of the sodium carbonate in the presence of free carbonic acid at alveolar tension must be in the form of bicarbonate.

d. Comparison of Oxalate and Hirudin Plasmas.—Blood from an arm vein was drawn into a tube containing a few flakes of hirudin, and the carbon dioxide capacity of the plasma was determined. Duplicate readings were 0.74 and 0.74 cc. of gas at 23° , 760 mm., indicating a capacity of 61.6 volume per cent of carbon dioxide bound by 1 cc. of plasma. In 5 cc. of the plasma 0.050 gm. (1 per cent) of potassium oxalate was dissolved, and the determinations were repeated. The readings were again 0.74 and 0.74 cc., showing that the oxalate had no measurable effect on the results.

This experiment is final proof that even twice as much oxalate as is used in our routine is without significant effect on the carbon dioxide capacity of the plasma.

Experiment II. Effect of Concentration of Free Carbonic Acid on the Amount Bound as Bicarbonate by Plasma.—In order to obtain evidence concerning the magnitude of the effect which changes in the carbon dioxide content of the air used for saturating would have on results of our plasma analyses, we have determined the amounts of carbon dioxide absorbed by plasma in equilibrium with atmospheres containing from 3 per cent of carbon dioxide upwards. The results are given in Table V and Fig. 4.

The plasma was a mixture of several samples from diabetic patients, some of whom showed slight degrees of acidosis. The mixture of plasmas showed about the minimum carbon dioxide capacity which we have observed in plasma from normal individuals. The determinations of carbon dioxide were performed on 1 cc. samples in the usual manner. Because of the variation in the carbon dioxide content of the saturating air, however, the results could not be calculated by the table at the end of this paper, but had to be reckoned by direct application of Equation 1 (p. 342). The barometer was 760 mm. and the temperature 27°. Under these conditions the constants of Equation 1 have the following values: $f = 0.827$; $\alpha_{\text{CO}_2} = 0.79$; $\alpha_{\text{air}} = 0.0165$.

Experiment III. Effect of Free Carbonic Acid on the Amounts Bound by Sodium Carbonate, Sodium Phosphate, and Sodium Albuminate.—These data form but the preliminary steps of a study of the carbon dioxide carrying mechanism of the blood, but they are presented here because they are at least suggestive of the manner in which carbonic acid reacts with the blood constituents.

Sodium Carbonate.—A 0.1 per cent solution (0.0188 N) of Merck's "reagent" Na_2CO_3 was saturated with carbon dioxide under varying tensions. The amounts of bound carbon dioxide were calculated, as in the case of the plasma in the preceding experiment, with the aid of Formula 1, which here, however, is altered by removal of the factor 0.975 from the last term, since such a dilute solution may be assumed to have practically 100 per cent of the dissolving power of water for gases, instead of the 97.5 per cent observed by Bohr in the case of plasma.

The experiment with sodium carbonate serves chiefly the purpose of a control of the methods as employed over a wide range of carbon dioxide tensions. As will be seen from the Na_2CO_3 curve of Fig. 4, the method gave practically theoretical results throughout the entire range of tensions, the Na_2CO_3 binding the amount of H_2CO_3 necessary to convert it into NaHCO_3 .

Sodium Albuminate.—4 gm. of Merck's egg albumin were dissolved in 25 cc. of water in a 50 cc. flask, and 0.5 cc. of a 1 per cent solution of phenolphthalein was added. A solution of N/7 sodium hydrate was then added from a burette until the albumin assumed the rose-color indicating a hydrogen ion concentration of approximately $10^{-8.5}$, which is about that of plasma from which the free carbonic

TABLE V.

Effect of Carbonic Acid Concentration on the Amount of CO₂ Bound as Bicarbonate by Plasma.

CO ₂ in air used for saturating plasma.	V. Vol. gas extracted from 1 cc. of saturated plasma.	Average V.	CO ₂ , reduced to 0°, 760 mm., in 100 cc. of plasma.			Molecular concentration of bi- carbonate.
			Total.	Dissolved as free car- bonic acid.	Bound as bicarbonate.	
<i>per cent</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	
3.2	0.627 0.622	0.625	53.4	2.1	51.3	0.0229
5.5	0.676 0.676	0.676	58.1	3.7	54.4	0.0243
Alveolar air.	0.681 0.691	0.686	59.0	3.7*	55.3	0.0247
9.6	0.740 0.740	0.740	64.0	6.4	57.6	0.0259
23.0	0.898 0.898	0.898	78.4	15.6	62.8	0.0280
100	0.779 0.779 (From 0.5 cc. of plasma.)	0.779	127.0	68.0	69.0	0.0308

* Calculated on the assumption that the alveolar air from the analyst's lungs contained the average normal of 5.5 per cent of carbon dioxide. It may, of course, vary several tenths of a per cent from this.

The apparent agreement of most of the duplicates to within 0.001 cc. on the readings is somewhat misleading. The readings were made to the nearest 0.005 cc. and corrected according to the calibration of the burette. Absolute apparent agreement, therefore, indicates agreement not necessarily closer than 0.005 cc., which is about as close as one can read the instrument with a slightly milky solution like diluted and acidified plasma.

acid has been pumped out. The solution was then diluted up to the 50 cc. mark. The concentration of albumin, 8 per cent, was approximately that of the proteins of the plasma. The concentration of sodium in the solution was 0.0143, sufficient to bind as bicarbonate about one-half the amount of carbon dioxide held by normal human plasma.

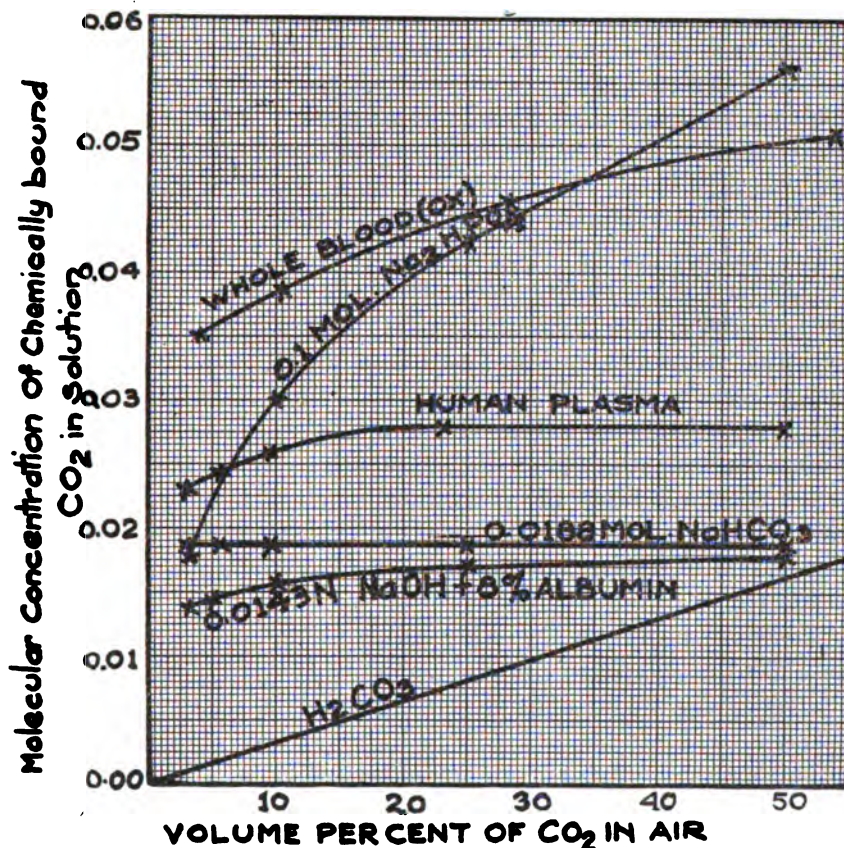


FIG. 4. Effect of free carbonic acid on the amounts bound as bicarbonate by plasma, and by sodium albuminate solution of alkali and protein concentrations similar to those of plasma.

Sodium Phosphate.—A tenth molecular solution of Na_2HPO_4 prepared according to Soerensen was used. The phosphate solutions require for the attainment of equilibrium with atmospheric carbon dioxide considerably more time than the 2 minutes' shaking which suffices for plasma and carbonate solutions. The phosphate solutions were accordingly shaken repeatedly with the gas mixtures,

and analyzed after each shaking, until constant results showed that the maximum amounts of carbon dioxide had been absorbed by the reaction $\text{H}_2\text{CO}_3 + \text{Na}_2\text{HPO}_4 = \text{NaHCO}_3 + \text{NaH}_2\text{PO}_4$.

It will be seen that the curve obtained with the albuminate solution closely resembles that obtained with plasma. In equilibrium with 5.5 per cent carbon dioxide in the air the solution binds almost exactly the amount of carbon dioxide that the sodium hydrate alone would combine with if no albumin were present. When the carbonic acid content of the solution is greatly increased the albumin also binds an appreciable though small amount of carbon dioxide, as is evidenced by the fact that more is taken up than the sodium hydrate alone could account for. This does not prove that in the plasma all the carbon dioxide binding power is due to alkaline carbonate and protein,—the plasma curve can also be almost exactly duplicated by a solution containing 0.01 M Na_2HPO_4 and 0.02 M NaHCO_3 . It is known, however, that the plasma contains only about 3 mg. of inorganic phosphorus per 100 cc. (Greenwald, 1915) which is too little to affect measurably the carbon dioxide capacity. Nor do ash analyses on the plasma indicate the presence of significant amounts of any other crystalloid buffers. The weight of evidence indicates that the only buffers of significance in the plasma are the proteins and the carbonates.

The whole blood, on the other hand, yields a constantly rising curve like the phosphate solution, indicating the participation of the relatively abundant phosphates of the corpuscles.

Experiment IV. Effect of Temperature Saturation on the Amount of Carbon Dioxide Bound by Plasma.

The determinations were made in the usual manner, except that in order to saturate at 10° and 40° the separatory funnels were shaken in baths at these temperatures. Short thermometers dipping into the plasma were placed inside the funnels, and the saturations were finished after the desired temperatures had been reached. Results were obtained with plasma from seven different individuals, and were calculated by means of Equation 1. The values for the solubilities of air and carbon dioxide used in the formula are, of course, partly the values for room temperature, partly for the temperature of saturation. The results are given in Table VI, and in the curves of Fig. 5.

The approximately linear form of the curves of Fig. 5, shows that the temperature effect between 10° and 40° is fairly constant. The maximum percentage decrease in carbon dioxide capacity caused by 1° rise in temperature was 0.47 per cent of the amount of carbon dioxide bound at 20°. The minimum was 0.25 per cent; the mean

TABLE VI.

Effect of Temperature on the Carbon Dioxide Binding Power of Human Plasma.

Plasma No.	Temperature of sat. ration.	Room temperature.	Barometer.	Vol. of gas observed.	CO ₂ per 100 cc. plasma.			Decrease in bound CO ₂ per 1° increase in temperature between 10° and 40°.	
					Total.	Dissolved as free carbonic acid.	Bound as bicarbonate.		
	°C.	°C.	mm.	cc.	cc.	cc.	cc.	cc.	per cent of CO ₂ bound at 20°
1	10	24.5	758	0.700	60.6	6.5	54.1	0.13	0.25
	24.5	24.5	759	0.650	56.3	4.1	52.2		
	40	24.5	758	0.605	52.8	2.7	50.1		
2	10	23.5	761	0.855	75.7	6.5	68.2	0.31	0.47
	23.5	23.5	761	0.780	69.2	4.2	65.0		
	40	23.5	761	0.701	62.3	2.7	59.6		
3	10	24	757	0.721	62.5	6.5	56.0	0.14	0.25
	24	24	757	0.671	58.4	4.2	54.2		
	40	24	757	0.623	54.6	2.7	51.9		
4	10	22	754	0.781	68.7	6.5	62.2	0.17	0.28
	22	22	754	0.721	63.4	4.5	58.9		
	40	22	749	0.762	60.0	2.7	57.3		
5	10	24	757	0.791	69.0	6.5	62.5	0.24	0.40
	24	24	757	0.711	62.5	4.2	58.3		
	40	24	757	0.662	58.1	2.7	55.4		
6	10	24	757	0.721	63.6	6.5	57.1	0.14	0.25
	24	24	757	0.671	58.6	4.2	54.4		
	40	24	757	0.623	55.6	2.7	52.9		
7	10	22	754	0.781	69.7	6.5	63.2	0.24	0.40
	22	22	754	0.721	63.0	4.5	58.5		
	40	22	748	0.682	58.9	2.7	56.2		

between the extremes is 0.36 per cent. The variation on both sides of the mean is large compared with the size of the mean value itself. As the entire temperature effect is so small, however, the mean temperature coefficient can be used over the range of ordinary room temperature without introducing significant errors.

The decrease in carbonic acid binding power caused by increase in temperature appears to result chiefly from the lowering of the solubility of carbon dioxide and the consequent decrease in the concentration of free carbonic acid in the experiments at the higher temperatures. Raising the temperature from 10° to 40° diminished the carbonic acid CO_2 from 6.5 volume per cent to 2.7. The average

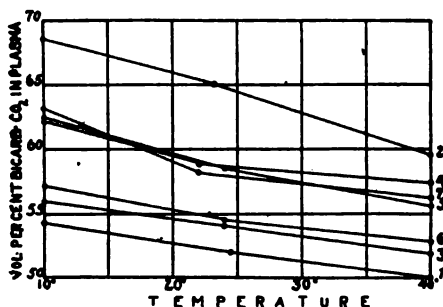


FIG. 5. Effect of temperature on the carbon dioxide capacity of plasma.

effect on the bicarbonate CO_2 in the seven plasmas was to lower it 6 volumes per cent. Interpolation on the curve of Fig. 4 indicates that in the experiment there tabulated a lowering of carbonic acid CO_2 from 6.5 to 2.7 volume per cent without any temperature change decreased the plasma bicarbonate 5.1 volume per cent, or nearly as much as when the change in free carbonic acid was accompanied by an increase of 30° in temperature. That temperature does influence the equilibria between carbon dioxide and buffers independently of its effect on carbon dioxide solubility is certain (Henderson, 1906), but the effect in the plasma appears to be slight compared with that of changing concentrations of free carbonic acid.

Experiment V. The Effect of Acids on the Carbon Capacity of Plasma.

β -Hydroxybutyric Acid.—Varying amounts of standardized solutions of Kahlbaum's hydroxybutyric acid were weighed into portions of a sample of human plasma, the concentrations of acid being such that the volume increase caused by its addition to the plasma was always less than 1 per cent. The plasmas were then saturated with 5.5 per cent carbon dioxide and analyzed.

Hydrochloric Acid.—Varying amounts of 0.1 N and 0.2 N HCl were added to 10 cc. portions of another plasma, with sufficient water in each case to bring the volume up to 12 cc. The mixtures were then saturated with 5.5 per cent CO_2 and analyzed.

The results with both acids are given in Table VII.

TABLE VII.

Acid.	Concentration of acid. Mols. per liter.	CO ₂ bound as bicarbonate.		Decrease in bicarbonate caused by acid. Mols. per liter.
		Vol. per cent.	Mols. per liter.	
Hydrochloric.	0.0000	58.0	0.0259	0.0000
	0.0042	48.5	0.0216	0.0043
	0.0083	41.3	0.0184	0.0075
	0.0167	25.3	0.0113	0.0146
	0.0250	12.5	0.0056	0.0203
	0.0333	2.8	0.0012	0.0247
	0.0500	0.0	0.0000	
β -Hydroxybutyric.	0.0000	67.6	0.0283	0.0000
	0.0096	43.3	0.0182	0.0101
	0.0240	23.9	0.0100	0.0183
	0.0481	3.7	0.0016	0.0267
	0.0962	0.0	0.0000	

Comparison of the first and last columns of the table shows that until acid equivalent to about half the plasma bicarbonate has been added the fall in bicarbonate approximately equals in molecular equivalents the amount of acid added. As the amount of acid becomes greater, however, the drop in plasma bicarbonate begins to fall short of the added acid. This is due to the fact that the H_2CO_3 concentration is kept constant, instead of being reduced in proportion to the bicarbonate. The condition is similar to that of the blood in uncompensated acidosis. The $\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}$ ratio, and consequently the

C_{π} , is increased. As a result the other plasma buffers (chiefly proteins) bind a measurably greater amount of acid than they could at normal C_{π} , and the acid so bound is prevented from decomposing bicarbonate.

The effect on the routine plasma determination is that the bicarbonate determined by our technique denotes a fall in the more severe stages of acidosis which is not quite so great as the actual drop in bicarbonate *in vivo*. The relationship between added acid and decrease in bicarbonate, however, is made so constant by saturating the plasma at a definite carbon dioxide tension that the lack of absolute numerical proportionality in the lower ranges is no practical detriment to the interpretation of results.

Experiment VI. Effect of Preservation on the Carbon Dioxide Capacity of Plasma.

A sample of oxalated human plasma was placed in a paraffin-lined tube in a refrigerator at approximately $+1^{\circ}\text{C}$. Samples were removed at intervals and analyzed with the following results.

TABLE VIII.

Age of plasma sample kept at $+1^{\circ}$ in paraffined tube.	Gas vol. read.	Temperature.	Barometer.	CO ₂ capacity. (Cc. of CO ₂ reduced to 0° , 760 mm., bound chemically by 100 cc. of plasma.)
				vol. per cent
days	cc.	$^{\circ}\text{C}$.	mm.	
0	0.70	21	750	56.8
1	0.71	19	750	57.6
2	0.71	21	753	57.8
4	0.70	22	752	57.0
6	0.70	22	752	57.1
12	0.68	27	767	56.7
20	0.61	20	764	49.4

For 12 days the capacity of the plasma remained stationary at 57.3 ± 0.5 volume per cent of carbon dioxide. The first change was noted in the 20 day sample, and was probably due to bacteria, as the samples for analysis were not drawn under sterile conditions. It may be well, however, to emphasize the fact that plasma kept in ordinary glass, instead of paraffin, may dissolve enough alkali over night to increase its carbon dioxide capacity by several per cent.

VII. Effects of Manner of Drawing Blood on the Carbon Dioxide Content of Whole Blood Samples, and the Carbon Dioxide Capacity of the Plasma.

Experiment VII, a. Blood from Different Veins Compared with Arterial. Effect of Momentary Exposure to Air.

An 18 kilo dog was etherized with a cone (no artificial respiration) and the femoral artery and vein of the right thigh, the brachial artery and vein of the left fore leg, and the left external jugular vein were exposed. Blood samples were drawn from each vessel both by means of a syringe, and of a needle connected with a 50 cc. Erlenmeyer flask. The latter was fitted with a two-hole rubber stopper through which passed two short glass tubes extending for 1 or 2 cm. both above and below the stopper. To one of the tubes was attached about 10 cm. of small bore rubber tubing, into the farther end of which was fitted the needle. To the other glass tube was attached a somewhat longer piece of rubber tubing by means of which suction could be applied to accelerate the flow of blood from the vein. As in the case of the McRae needle, the blood was drawn with moderate suction into an open vessel, and fell through a height of several centimeters, so that for 0.01 second or less opportunity was given for escape of venous carbon dioxide.

Blood was allowed to flow into syringes simultaneously from the femoral and jugular veins. Then the syringes were immediately replaced by two collecting flasks of the kind described above, and samples of blood were drawn into them. Consequently these four samples may be considered as practically simultaneous.

The same procedure was then repeated with the femoral artery, brachial vein, and brachial artery. The entire operation, from beginning of anesthetization through the drawing of the second set of samples, consumed 30 minutes. Samples of the whole blood were analyzed for carbon dioxide content at once, the technique described on p. 389, of the succeeding article being employed. Other samples were meanwhile centrifuged, and the carbon dioxide capacity was determined, as described in this paper, after saturating at 20° with air containing 5.5 per cent of carbon dioxide. The results are given in Table IX.

The results may be summarized as follows:

1. Blood samples drawn without loss of carbon dioxide (syringe samples) within an interval of a few minutes from three different veins showed within 2.5 per cent the same carbon dioxide contents.
2. The carbon dioxide content of the venous blood from the resting dog (the animal was anesthetized without a struggle) was 2 to 5 per cent greater than that of the arterial blood. Blood drawn from

TABLE IX.

- (1) *Carbon Dioxide Content of Blood from Different Veins Compared with Arterial.*
 (2) *Effect of Manner of Drawing Blood on CO₂ Content of Whole Blood and CO₂ Capacity of Plasma.*

		Drawn simultaneously.		Drawn simultaneously a few minutes later.		
		Femoral vein.	Jugular vein.	Brachial vein.	Brachial artery.	Femoral artery.
Vol. per cent of CO ₂ in whole blood as drawn.	Drawn with syringe (no loss of CO ₂).....	47.6	46.1	45.1	42.1	43.1
	Blood stream falling through air in receiving flask.....	43.6	44.1	42.1	41.6	41.1
	Difference.....	4.0	2.0	3.0	0.5	2.0
Carbon dioxide capacity of plasma from above blood samples.	Plasma from blood drawn with syringe.....	63.1	60.9	60.9	56.6	58.0
	Plasma from blood drawn into flask.....	57.1	58.0	58.0	55.1	57.1
	Difference.....	6.0	2.9	2.9	1.5	0.9

the brachial vein showed 3 per cent more carbon dioxide than blood drawn simultaneously from the brachial artery.

3. The effect of drawing the blood into an open flask in which the blood stream was allowed to fall through the air for several centimeters was to cause a loss of carbon dioxide from the venous blood such as to bring its CO₂ content to approximately that of arterial. The carbon dioxide held by the venous blood in excess of that of the arterial appears to be given off readily, so that the instant's exposure while the blood was falling into the receiving vessel approximately transformed venous into arterial blood, so far as the carbon dioxide was concerned.

4. The CO₂ capacity of the plasma rose and fell parallel with the CO₂ content of the whole blood from which the plasma was centrifuged. The carbon dioxide bound chemically by plasma saturated at 20° with air containing 5.5 per cent of CO₂ amounted to 15 ± 1 volume per cent more than the total carbon dioxide content of the whole blood at the time it was centrifuged. Within the limits of *this*

experiment (compare Experiment VII, *c*), differences in the CO₂ content of the whole blood at the time of centrifugation resulted in approximately equal differences in the carbon dioxide capacity of the plasma, so that the latter remained at a level approximately 15 volume per cent greater than the former. The effect of conditions at the time of centrifugation on the bicarbonate content of the plasma separated is to be attributed to the transfer of electrolytes between plasma and corpuscles under the influence of changing free carbonic acid concentration, discussed on p. 331.

Experiment VII, b. Comparison of Blood Samples Drawn with Syringe and McRae Needle.

The experiment was performed like the preceding, except that for the samples drawn in open vessels a McRae needle was used, so that the conditions entirely simulate those obtained with the use of this apparatus for clinical purposes.

TABLE X.

Carbon Dioxide Contents of Whole Blood Samples from Normal Dog. 1. From Different Blood Vessels. 2. Drawn by: (a) Syringe, without Loss of Carbon Dioxide. (b) McRae Needle in Open Tube.

Vessel.	Vol. per cent of carbon dioxide in blood.			
	Immediately after being drawn.		After standing 6 hours in partly filled tube closed only by cotton plug.	
	Syringe.	McRae needle.	Syringe.	McRae needle.
Femoral vein.....	37.1	33.0	30.4	30.3
Brachial vein.....	35.2	32.4	—	30.8
External jugular vein.....	37.1	—	28.9	—
Femoral artery.....	34.0	29.9	26.1	—

The results confirm those of the preceding experiment. In addition they show that whole blood standing in a partly filled tube may lose in 6 hours up to 8 volume per cent of carbon dioxide.

Experiment VII, c. Effect of Different Methods of Drawing Samples on Results Obtained with Venous Blood Heavily Charged with Carbon Dioxide as the Result of Exertion.

The dog used in this experiment exerted himself strenuously against etherization, and consequently the carbon dioxide content of the venous blood was 10.2 volume per cent greater than that of the arterial, instead of the 3 to 5 per cent difference usually noted. The samples were all collected within an interval of a few minutes.

TABLE XI.

		Right jugular vein.	Left jugular vein.	Femoral artery.
		vol. per cent	vol. per cent	vol. per cent
CO ₂ in whole blood as drawn.	{ Drawn with syringe (no loss of CO ₂).....	42.5	42.1	32.0
	{ Drawn with McRae needle (blood stream falling through air).....	28.6	30.6	25.8
CO ₂ bound as bicarbonate by plasma from above blood samples. Plasma is saturated with air containing 5.5 per cent CO ₂ .	{ Plasma from blood drawn with syringe.....	52.6	51.6	44.9
	{ Plasma from blood drawn with McRae needle.....	47.9	46.8	44.0

The results confirm those of the foregoing experiments in showing that allowing the blood to fall for a few centimeters through air when the sample is drawn (with a McRae needle) reduces it to approximately the carbon dioxide content of arterial blood. The fact has additional interest in this case, because the difference between arterial and venous bloods is, presumably on account of the animal's exertions, two or three times as great as that usually observed in resting dogs. Nevertheless the instant's exposure of the falling blood removed all the carbon dioxide in excess of that in the arterial blood, and even somewhat more. The result, however, was, as in the foregoing experiments, to bring the samples drawn with momentary

exposure to air closer to arterial blood in carbon dioxide content than were samples of venous blood drawn without exposure.

Comparison of the carbon dioxide contents of the different whole blood samples and of the carbon dioxide binding powers of the corresponding plasmas affords more examples of the influence of carbonic acid on the acid-base transfer between corpuscles and plasma. Samples of whole blood from which some of the carbon dioxide escaped, during the momentary aeration connected with the use of the McRae needle, yielded plasmas which had also a reduced amount of base capable of binding carbonic acid.

Experiment VII, d. Collection of Blood under Paraffin Oil without Loss of Carbon Dioxide.—The following experiment shows that with centrifuge tube and needle arranged as shown in Fig. 1, one can collect blood samples without appreciable loss of carbon dioxide. The blood in entering the centrifuge tube mixes with the finely powdered oxalate, so that very little additional stirring is necessary in order to prevent clotting. After a sample was drawn the stopper was loosened, and the blood was stirred gently with the inlet tube. Previous experience had shown that if the mixing of the oxalate were attained by more vigorous agitation, such as shaking the tube or turning it upside down repeatedly, a measurable loss of carbon dioxide would occur, even if the layer of oil separating the blood from the air was not broken. Since carbon dioxide is more soluble in the oil than in the water, vigorous agitation of the two fluids results in a partial transfer of carbon dioxide from water to oil. The latter, however, prevents rapid diffusion of the gas away from the surface of the water layer, and if unnecessary agitation is avoided, this form of tube yields results identical with those obtained with syringe samples. The chief advantages over the syringe are in cost, and in the convenience of having the needle on a flexible connection.

The dog used, a female bull terrier of 16 kilos weight, was etherized with the Meltzer-Auer insufflation apparatus. The animal submitted to etherization very quietly; the resting condition is evidenced by the fact that there is only 3 per cent difference in carbon dioxide content between arterial and venous bloods.

It will be noted that the figures in the last column afford another illustration of the effect of CO_2 tension on the acid-base transfer between corpuscles and plasma.

TABLE XII.

Collection of Blood under Paraffin Oil without Loss of Carbon Dioxide.

Blood vessel from which sample was taken.	Instrument used in taking samples.	Total CO ₂ content of blood drawn.	CO ₂ bound as bicarbonate by plasma saturated at 20° with 5.5 per cent CO ₂ .
		<i>vol. per cent</i>	<i>vol. per cent</i>
Right jugular vein.	Syringe.	33.0	41.3
	Oil tube.	33.0	41.0
	McRae needle.	29.3	32.9
Left jugular vein.	Syringe.	33.9	41.3
	Oil tube.	33.9	41.3
	McRae needle.	33.0	39.6
Femoral artery.	Syringe.	30.3	37.6
	Oil tube.	30.0	37.1
	McRae needle.	25.6	33.3

Experiment VII, e. Effect of Manner of Drawing Blood on Results Obtained from Normal Human Subject.

A sample of blood was drawn from the right arm with a syringe, care being taken to avoid any chance for loss of CO₂. About 1 minute later a sample was taken from the other arm, conditions being the same except that the McRae needle was used in this case. In each case the arm was ligated with a rubber band for about 1 minute before the sample was drawn. The analyses yielded the results shown in Table XIII.

Comparison of analyses *a* and *b* shows, as in the foregoing dog experiments, that use of the McRae needle results in loss of some of the excess carbon dioxide of the venous blood, so that the venous blood is brought closer to arterial (analyses *b*) in its carbon dioxide content.

Comparison of *c* and *d* shows that the approximation of the results to the arterial standard, as a result of the momentary aeration connected with use of the McRae needle, is also noted when the carbon dioxide capacities of the plasmas are considered. The plasma from the venous blood drawn with the McRae needle approximated the plasma from arterialized blood in its carbon dioxide capacity.

TABLE XIII.

Comparison of Blood Samples Drawn from Normal Man with Syringe and McRae Needle.

		Carbon dioxide.
		<i>vol. per cent</i>
a. CO ₂ content of whole blood as drawn.	Syringe.	67.7
	McRae needle.	62.1
b. CO ₂ content of whole blood arterialized by shaking at 37° with air containing 5.5 per cent of CO ₂ .	Syringe.	57.2
	McRae needle.	57.2
c. CO ₂ bound as bicarbonate by plasma centrifuged from freshly drawn blood and saturated with 5.5 per cent CO ₂ at 23°.	Plasma from syringe sample.	76.3
	Plasma from McRae needle sample.	73.3
d. CO ₂ bound as bicarbonate by plasma centrifuged from arterialized blood (analyses b above) and resaturated at 23° with 5.5 per cent CO ₂ .	Plasma from arterialized syringe sample.	71.0
	Plasma from arterialized McRae needle sample.	71.5

Experiment VIII. Demonstration of Identical Bicarbonate Contents of Venous and Arterial Bloods under Identical Carbon Dioxide Tensions.

A dog of 24 kilos weight was placed under ether with the Meltzer-Auer apparatus and blood samples were drawn as indicated in the following table. Each blood sample was divided into three portions. In one the carbon dioxide content was determined directly (third column). In another the carbon dioxide was determined after saturation of 37.5° of the fresh blood with air containing 5.5 per cent of carbon dioxide. It will be noted that this treatment raised the carbon dioxide of the arterial blood by 10 volume per cent, indicating that the arterial carbon dioxide tension of this animal, presumably because of the artificial ventilation, was considerably less than that of the average man. A third sample of each blood was centrifuged immediately and the plasma bicarbonate CO₂ was determined in the routine way, after saturation of the plasma at room temperature with 5.5 per cent CO₂ in air.

Experiment VII, *d*, is confirmed in showing that blood can be drawn into the "oil tube" without measurable loss of carbon dioxide. The results obtained are identical with those from samples drawn with the syringe.

TABLE XIV.

Blood vessel.	Instrument used in drawing blood.	Total CO ₂ content of whole blood as drawn.	Bicarbonate CO ₂ of plasma after saturation at 20° with air containing 5.5 per cent CO ₂ .	Total CO ₂ content of whole blood after saturation at 37° with air containing 5.5 per cent CO ₂ .
		<i>vol. per cent</i>	<i>vol. per cent</i>	<i>vol. per cent</i>
Left jugular vein.	Syringe.	38.9	48.2	—
		39.3	48.2	—
	Oil tube.	39.3	48.2	46.8
		39.3	48.2	45.9
Left femoral artery.	Oil tube.	36.4	44.5	46.7
		36.4	44.5	45.9

The figures in the last column show that when arterial and venous bloods are brought to the same content of free carbonic acid, the bicarbonate contents are also equal. From this it follows that blood in passing from arteries to veins in the resting animal does not take up sufficient acid other than carbonic to affect the bicarbonate content appreciably. So far as the content in non-volatile acids is concerned, there is no appreciable difference in the resting animal between blood from the arteries and that from the jugular vein.

The figures in the middle column illustrate again the effect of carbonic acid concentration on the distribution of bases and acids between plasma and corpuscles. Plasma centrifuged from venous blood, with 3 per cent more total carbon dioxide than arterial, showed a bicarbonate CO₂ nearly 4 per cent higher than the arterial plasma.

Experiment IX. Effect of Experimental Acidosis on the Carbon Dioxide Figures and the Hydrogen Ion Concentration of Venous and Arterial Blood.

The animal used was a collie bitch of 14.5 kilos weight, in splendid condition.

At 2.00 p.m. the animal was etherized, and the femoral veins and arteries on both sides were exposed. Ether anesthesia was maintained throughout the experiment by the Meltzer-Auer insufflation method.

At 2.15 blood samples of about 15 cc. each were taken from the right femoral artery and the left femoral vein, the samples being collected under paraffin oil with precautions to prevent loss of carbon dioxide (see p. 334).

At 2.20 the injection of $N H_2SO_4$ from a burette into the right femoral vein was begun.

At 3.00 50 cc. had been injected. The animal showed marked dyspnea and a pulse of 180. These symptoms disappeared after 10 minutes, and the injection was resumed.

At 3.30 75 cc. of $N H_2SO_4$ in all had been injected, and the injection was concluded.

At 4.10 a second pair of blood samples was taken, the vessels used being the right femoral artery and the left femoral vein.

At 5 two more samples were taken, in order to ascertain whether the animal's blood indicated that she was overcoming the acidosis.

The hydrogen ion concentrations were determined in a Clark electrode (Clark, 1915), successive portions of the same sample being shaken in the same hydrogen atmosphere until the latter had acquired the carbon dioxide tension of the blood, according to the principle of Hasselbalch's technique (Hasselbalch, 1911, 1913). The figures obtained consequently may be taken as representing the actual hydrogen ion concentration of the blood in the veins and arteries.

The gas analyses of whole blood and plasma were performed as described in this and the succeeding paper.

The results are given in Table XIV. Graphic comparison of the carbon dioxide figures is given in Fig. 6.

TABLE XV.
Effect of Acid Injection on Blood.

	Whole blood as drawn.				Plasma after saturation at 20° with air containing 5.5 per cent CO_2 .	
	Arterial.		Venous.		Arterial.	Venous.
	Total CO_2 .	Re-action.	Total CO_2 .	Re-action.		
	vol. per cent	pH	vol. per cent	pH	vol. per cent	vol. per cent
Before injection.....	38.7	7.33	47.9	7.28	49.9	53.2
40 min. after injection of 75 cc. $N H_2SO_4$	10.1	—	22.1	7.23	17.4	27.7
90 min. after injection.....	10.1	7.17	22.1	7.17	16.5	26.8

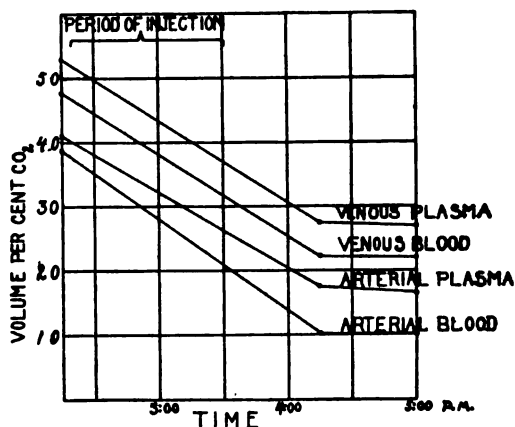


FIG. 6. Effect of acid injection on blood and plasma.

Discussion of the Results of Experiment IX.

1. The curves of Fig. 6 show that in acidosis the arterial⁷ carbon dioxide is approximately paralleled in its fall by the other three values determined. For reasons discussed on p. 323 the arterial bicarbonate, or the nearly identical total arterial carbon dioxide, is the ideal figure to determine as a measure of the alkaline reserve of the body. The curves of Fig. 6 indicate, however, that any one of the other three values may also be used as an index of the alkaline reserve, provided the limits of the normal level, and the changes therefrom corresponding to different grades of acidosis, are determined for the value used. The necessity for employing in all clinical and much experimental work one of the values obtained on the venous blood rather than the theoretically preferable arterial figure is, of course, obvious. The value obtained by the technique for acidosis study outlined in this paper, *viz.*, the capacity of the venous plasma to combine with carbon dioxide, has been given the preference in our work chiefly for practical reasons, such as the ease and convenience of making determinations on the plasma as compared with whole blood, the facts that plasma can be preserved for days and even weeks and still show unchanged carbon dioxide binding power when resaturated with 5.5 per cent CO_2 , and that unlike the carbon dioxide

capacity of the whole blood the carbon dioxide capacity of the venous plasma maintains its parallelism with the arterial carbon dioxide even in the severe grades of acidosis (Stillman, Van Slyke, Cullen, and Fitz).

2. The greater part of the injected acid did not remain in the blood, but was at once transferred elsewhere and presumably neutralized by the bicarbonate and phosphate reserves in other parts of the body. The amount of blood in a 14 kilo dog may roughly be estimated at 1 liter. The injection of 75 cc. of N acid into this volume of bicarbonate solution would decompose sufficient bicarbonate to reduce its molecular concentration by 0.075. The actual reductions of carbonate noted as the result of the acid injection were the following, the figures being transposed from terms of volume per cent carbon dioxide to molecular concentration:

Arterial whole blood CO ₂ content, reduced	0.0126 M.
Venous " " " " "	0.0115 "
Arterial plasma, CO ₂ capacity,	0.0104 "
Venous " " " " "	0.0119 "

As bicarbonate furnishes about nineteen-twentieths of the CO₂ of the whole blood, only one-twentieth being free carbonic acid, the whole blood figures as well as those of the plasma (where the CO₂ from H₂CO₃ is subtracted) may be taken as practically bicarbonate figures. The fall, on the average only 0.012 in molecular concentration of both whole blood and plasma, indicates that only about one-sixth of the injected acid remained in the blood, or was neutralized by the blood bicarbonate. The quick transfer between blood and tissues indicates that an acid-base equilibrium is continually maintained between them, even when there is such an enormously rapid influx of acid as occurred in this experiment. There is consequently direct experimental basis for assuming that the bicarbonate concentration of the blood is an index of the alkaline reserve of the entire body.

3. An actual increase in the hydrogen ion concentration of both arterial and venous blood occurred during the experiment. The acidosis was therefore partly uncompensated. Respiration did not lower the free carbonic acid enough to compensate entirely for the lowered bicarbonate, and the failure to do so is evidenced by an in-

crease in the hydrogen ion concentration (fall in pH) past the extreme normal limits. The failure may, however, be due to the effect of etherization on the respiratory control rather than to the reduction of arterial bicarbonate to one-fourth its previous level. Even severely sick patients appear able to compensate nearly if not quite so severe a grade of acidosis (Peabody, 1914). Michaelis and Davidoff (1912) have found, however, that narcosis of itself causes the respiratory centers to become less responsive to increase in hydrogen ion concentration, and consequently allow free carbonic acid to accumulate until the $\frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3}$ ratio, and consequently the hydrogen ion concentration, is considerably above normal. Evidence that the etherization was responsible in this experiment is seen in the fact that a further decrease in pH (increase in hydrogen ion concentration) occurred between the second and third bleedings when no acid was injected.

4. The injection of acid apparently results in an increase in the gas exchange occurring with each circulation of the blood. Before the injection the difference in CO_2 content between arterial and venous blood was 9 per cent; after the injection it had increased to 12 per cent. Similar increases in the gas exchange have been noted in other experiments after acid injection. In this case the increase in carbon dioxide occurring as the blood traverses the tissues cannot be attributed to CO_2 set free in the latter by the injected acid, for it was still undiminished $1\frac{1}{2}$ hours after the injection had been finished. The increased gas exchange must be due either to increased rate of oxidation in the tissues, or to decreased rate of blood flow. The problem suggested by this observation, *viz.*, whether acidosis, compensated or uncompensated, produced either experimentally or by disease, results in an increased rate of oxidation, we hope to attack later.

5. The effect of carbon dioxide on the acid-base transfer between corpuscles and plasma is again seen when the bicarbonate CO_2 figures for venous and arterial plasmas are compared. The bicarbonate contents of the sets of plasmas from the two sources differ by nearly the same margins as the total CO_2 contents of the respective whole bloods (see Fig. 6). The results obtained in the experiments of

sections VII and VIII show the same relationship between venous and arterial blood and plasma. Consequently the carbonic acid entering the blood from the tissues, during the passage from arteries to veins, must displace nearly an equivalent amount of other acid, which passes into the cells, leaving the plasma at its normal alkalinity. At least part of the acid thus displaced by combined mass action of the carbonic ($\text{H}_2\text{CO}_3 + \text{NaCl} = \text{NaHCO}_3 + \text{HCl}$) and diffusion is hydrochloric. The results shown in the next experiment indicate that this reaction is the one chiefly concerned when the carbonic acid concentration changes only within the limits likely to occur in the body. The physiological effect of the acid transfer into and out of the cells is to increase tremendously the power of the plasma to take up carbon dioxide without having its hydrogen ion concentration raised above the normal limits. The height to which it is possible to force the plasma bicarbonate by increasing the carbonic acid of the whole blood is indicated by the next experiment.

Experiment X. Effect of Free Carbonic Acid on the Acid-Base Equilibrium between Plasma and Corpuscles.

Blood was drawn with a McRae needle from the arm vein of a healthy man, and the sample was divided into four portions. One was centrifuged at once, and the others were shaken each with 100 volumes of atmosphere containing the proportions of carbon dioxide indicated in Table XVI. Carbon dioxide in the blood plasma was determined in the usual manner, chlorides by the method of McLean and Van Slyke (1915). The experiment reported here is one of a number which yielded similar results. The relationship between chloride and carbonate concentrations is made readily evident by Fig 7.

For furnishing the blood used in this experiment and performing the chloride determinations we are indebted to our colleague, Dr. Franklin McLean.

The figures for molecular concentration in the plasma of untreated blood (top line of Table XVI) show the relative importance of bicarbonate among the plasma electrolytes. It is, as pointed out by Henderson (1908, *b*), second only to the sodium chloride, the concentration of the bicarbonate being one-fourth to one-third that of the chloride. As shown by Hamburger, the average blood plasma is isotonic with a 0.9 per cent (0.155 molecular) solution of sodium chloride. The average chloride concentration may be taken, as in this blood, at about 0.105 molecular, the bicarbonate at 0.030 M,

the two together therefore at 0.135 M, leaving only about 0.020 M, or one-seventh the total, to be made up of all the other electrolytes in the plasma.

On examining the results from the three blood portions in which the carbonic acid was arbitrarily changed it becomes evident that a part of the resulting changes in plasma bicarbonate are explainable

TABLE XVI.

Effect of Free Carbonic Acid Concentration on Distribution of Chloride and Bicarbonate between Plasma and Corpuscles.

Treatment of whole blood before centrifugation.	Calculated free carbonic acid dissolved in blood as result of treatment.		Bicarbonate of plasma centrifuged from treated blood, then saturated at 20° with 5.5 per cent CO ₂ .		Plasma chloride.		Changes in plasma concentration caused by treatment of whole blood. Mol. concentration.	
	Vol. per cent CO ₂ .	Mol. concentration.	Vol. per cent CO ₂ .	Mol. concentration.	Calculated as gm. NaCl per liter.	Mol. concentration.	Bicarbonate.	Chloride.
No treatment, blood centrifuged as drawn..	3.0*	0.0013	69.8	0.0311	6.13	0.1050	—	—
Shaken with 100 volumes of air.....	0	0	42.2	0.0188	6.42	0.1098	-0.0123	+0.0048
Shaken with 5.5 per cent CO ₂ at 20°, 760 mm...	5.3	0.0024	77.9	0.0357	5.95	0.1017	+0.0046	-0.0033
Saturated with pure CO ₂ gas at 20°, 760 mm....	86.5	0.0386	135.8	0.0607	5.48	0.0939	+0.0296	-0.0111

* Blood as drawn assumed to be saturated with 5.5 per cent CO₂ at 37°, 760 mm.

by the migration of HCl from plasma into corpuscles. Changes within the limits of physiological possibility, such as that caused by changing the carbonic acid CO₂ from 3.0 to 5.3 volume per cent of the blood, may be chiefly accounted for by this shift in hydrochloric acid. When the carbonic acid is greatly altered other electrolytes also become involved in the transfer, for the changes caused by saturating the whole blood with either pure carbon dioxide or with air practically free of carbon dioxide are, in molecular equivalents, only about one-third as great in the plasma chloride as in the bicarbonate (see last column of Table XV).

In connection with the technique for determining the plasma bicarbonate, these results, showing the extreme possible effects of the acid-base transfer, indicate the magnitude of the changes in plasma which it is possible to cause by varying the carbon dioxide tension of the whole blood at the time the plasma is separated from the cells. Although these extreme effects greatly exceed those which could be caused by accidental loss or gain of carbon dioxide in handling blood samples, they serve to emphasize the importance of this

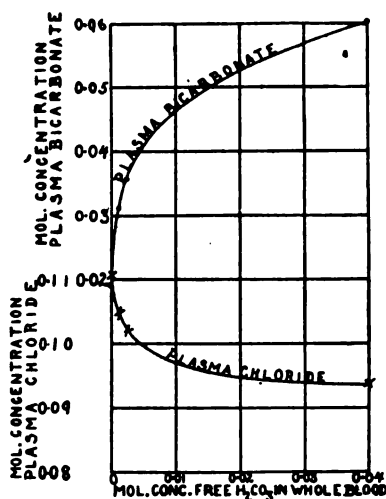


FIG. 7. Effect of free carbonic acid concentration on the distribution of chloride and bicarbonate between corpuscles and plasma. Each square, ordinate or abscissa, represents a change of 0.01 in molecular concentration.

possible source of error, and the necessity of observing the precautions for handling blood samples given on pages 333 and 334.

Incidentally the data also indicate the effect which carbonic acid changes in whole blood may have on the chloride content of the plasma. The effect in proportion to the total chloride is only one-third less than the relative effect on the bicarbonate, and if unrecognized could readily become a factor in the results of plasma chloride determinations.

SUMMARY.

Reasons are discussed for basing both the definition of acidosis and the methods for its detection on the blood bicarbonate.

Experiments are detailed showing both *in vivo* and *in vitro* the influence on the plasma bicarbonate of various factors, in particular of the shift of bases and acids between plasma and corpuscles under the influence of changing carbonic acid concentration.

A simple technique has been developed by means of which the capacity of the plasma to combine with carbonic acid under definite tension is determined as a measure of the alkali in excess of acids other than carbonic. The plasma, from oxalated blood, drawn and centrifuged under definite conditions, is shaken at room temperature in a separatory funnel filled with alveolar air from the lungs of the operator, or with an artificial air mixture containing 5.5 per cent of carbon dioxide. The carbon dioxide content of the plasma is then determined by the method described in the next paper. The results are calculated in terms of bicarbonate with the aid of the table on p. 344. The value determined appears to indicate not only the alkaline reserve of the blood, but also that of the entire body.

The results obtained with a given plasma are reproducible to within 1 volume per cent of CO₂, 65 volume per cent being the average normal value for man. In acidosis the carbon dioxide capacity of the plasma falls so far below normal that the method is a most sensitive indicator of this condition and its severity. The simplicity of the technique and the few minutes required for the determination make it available, not only for physiological experiments, but also for clinical routine. Results obtained with normal men and diabetic patients are given in Papers V and VI.

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STUDIES OF ACIDOSIS.

II. A METHOD FOR THE DETERMINATION OF CARBON DIOXIDE AND CARBONATES IN SOLUTION.*

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Previous methods for the determination of carbon dioxide in blood have been based chiefly on procedures for either boiling the carbon dioxide out of solution under diminished pressure, or for measuring the gas pressure resulting from addition of acid to the blood in a closed chamber (methods of Haldane and Barcroft).

The chief difficulties encountered in attempting to extract the carbon dioxide from water solution under diminished pressure and measure the gas have been the difficulty of complete extraction and the readiness with which the carbon dioxide is reabsorbed by the water as soon as the vacuum is released. In order to avoid these difficulties recourse has been had to boiling under diminished pressure.¹ The boiling drives out the gas completely from solution, so that it can be collected in a separate chamber and measured over mercury. The process, however, necessitates complicated apparatus, is time-consuming, and is likely to prove troublesome on account of the foaming of such a viscous mixture as blood. This method has, in practice, therefore been completely displaced by the Barcroft-Haldane methods with their relative rapidity and simplicity.²

* A preliminary report of the work was published in *Proc. Soc. Exp. Biol. and Med.*, 1914-15, xii, 165. A description of the use of the same apparatus for oxygen determination in blood was reported in *ibid.*, 1917, xiv, 84.

¹ Abderhalden, E., *Handb. biochem. Arbeitsmethoden*, Berlin, 1910, iii, 678-682.

² Barcroft, J., and Haldane, J. S., *J. Physiol.*, 1902, xxviii, 233. Barcroft, J., and Higgins, H. L., *ibid.*, 1911, xlii, 512.

The vacuum extraction principle has advantages, however, as recently demonstrated by Swanson and Hulett, in the short time within which, under low pressure, equilibrium is attained between the gas contents of water and atmosphere, and in the fact that the gases are obtained for direct volumetric measurement.³

In their method complete extraction of dissolved gases is not attempted. The solution is shaken at room temperature in an evacuated chamber, where the volume relations between the liquid and the free space are known. A definite proportion of each gas escapes into the free space, and the residue remaining in solution is calculated from the solubility coefficient of the gas. To prevent reabsorption of the gases, Swanson and Hulett transfer them to a burette without releasing the vacuum.

To simplify the application of the same principle to blood analysis and make the entire operation possible with a one-piece apparatus, we have varied the technique by removing the water instead of the gas from the chamber after the extraction, so that the gases can be measured over mercury in a calibrated tube at the top of the same chamber.

*Apparatus.*⁴

The apparatus as shown in Fig. 1 is designed in proportions especially adapted to determination of carbon dioxide in blood plasma. It consists essentially of a 50 cc. pipette with three-way cocks at top and bottom, and a 1 cc. scale on the upper stem, divided into 0.02 cc. divisions. At the bottom the apparatus is connected by a

³ Swanson, A. A., and Hulett, G. A., *J. Am. Chem. Soc.*, 1915, xxxvii, 2490. The use of the principle in the development of the present method is due directly to Professor Hulett's suggestion.

⁴ The apparatus is manufactured by the Emil Greiner Company, 55 Fulton St., New York.

Caution in Setting up Apparatus.—The jaws of the strong clamp in which the 50 cc. pipette of the apparatus is held should be lined with thick, soft pads of rubber. The pipette has to be clamped firmly, because of the weight of the mercury.

In order to prevent accidental slipping of the apparatus from the clamp, an iron rod of 6 or 8 mm. thickness should be so arranged as to project under cock / between *c* and *d*.

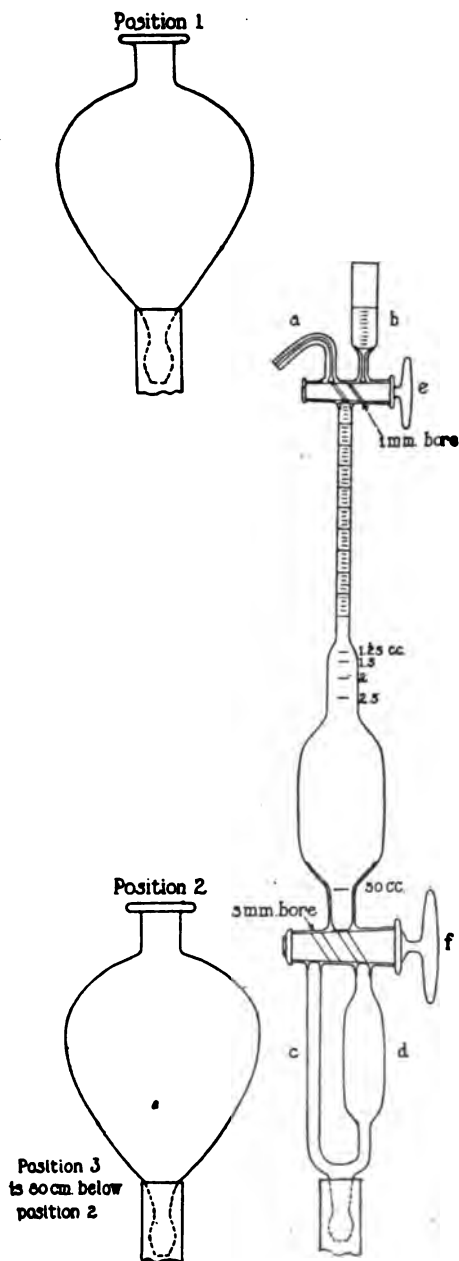


FIG. 1.

heavy walled rubber tube with a levelling bulb filled with mercury. The chamber *d*, serves to draw off the solutions, as above mentioned, after the carbon dioxide has been extracted from them, the other bottom connection *c*, serving for subsequent release of the vacuum by the entrance of mercury. The apparatus is made of strong glass, in order to stand the weight of mercury without danger of breaking, and is held in a strong screw clamp, the jaws of which are lined with thick pads of rubber. Capillary *a* is used for convenient removal of solutions from the apparatus. It may also for special gas analyses be used to connect the apparatus with an absorption pipette. Three hooks or rings at the levels 1, 2, and 3 serve to hold the levelling bulb at different stages of the analysis.

The calibrated upper stem of the pipette is of such diameter that 1 mm. of length corresponds to about 0.01 cc. By estimating tenths of a 0.02 cc. division, gas volumes can be read to 0.002 cc.

In order to justify such reading the apparatus must be accurately calibrated. To calibrate it one attaches to the outlet at the bottom, a short glass tube drawn out into a capillary tip, and fills the apparatus by suction with distilled water as far as the bottom of cock *e*. By manipulation of cock *f* the water is drawn off, 0.1 cc. at a time, into a weighing bottle, and weighed to within 1 mg.

It is essential that the cocks, especially *f*, shall be held in place so that they cannot be forced out by pressure of the mercury. For this purpose rubber bands may be used, but we have found elastic cords of fine wire spirals applied in the same manner as rubber bands to be stronger and more durable.

The Determination.

Outline.—Briefly stated, the 50 cc. pipette of the apparatus being full of mercury, the solution to be analyzed is acidified within the pipette, the total volume of water admitted being preferably 2.5 cc. A Torricellian vacuum is then obtained in the pipette by lowering the levelling bulb. The carbon dioxide is extracted from the water by a half minute's shaking in the evacuated chamber, and the water is drawn out of the 50 cc. chamber into *d*. Mercury is then readmitted through *c*, and the volume of gas is read at atmospheric pressure in the

finely graduated upper stem of the pipette. The observed volume is corrected by subtraction of the amount (0.04 to 0.05 cc., according to temperature) of air which enters the apparatus dissolved in the water, and by addition of the 4 to 5 per cent of the total CO_2 which remains unextracted because of its solubility in water. These corrections can be determined directly, as will be described, but can be calculated so accurately from the known solubilities of air and carbon dioxide in water that ordinarily only the single reading of the volume of gas first extracted is necessary, the result being calculated by a factor which includes all the corrections.

Testing the Apparatus.—Before a determination is made, the entire apparatus, including the capillaries above the upper cock, is filled with mercury. To test the apparatus for tightness and freedom from gases the mercury bulb is lowered to position 3, so that a Torricellian vacuum is obtained, the mercury falling to about the middle of *d*. The levelling bulb is then raised again. If the apparatus is tight and gas-free the mercury will refill it completely and strike the upper cock with a sharp click. In case there is any gas in the apparatus it serves as a cushion; the click is not heard, and a bubble remains above the mercury. If this is the case, the apparatus must be repeatedly evacuated until the gas has all been removed. Before the apparatus has been used, the rubber tubing, and even the glass walls, hold measurable amounts of gases, which are given off when the apparatus is evacuated. After it has once been freed from these gases, however, it can be used indefinitely without further trouble from this source if no air is admitted again. It is always desirable, nevertheless, before making the first determination of a series, to test the apparatus as above described.

Determination.—The apparatus, including both capillaries above the upper cock, is entirely filled with mercury, and the cup at the top washed free of acid with carbonate-free ammonia (see "Remarks on details" below). The solution to be analyzed is then run from a pipette into the cup. When the solution, like plasma, contains some free carbonic acid as well as carbonate, the tip of the pipette must dip below the surface of the solution in the cup during the transfer. If the liquid were allowed to run through the air in a free stream, carbon dioxide would escape from it. The apparatus is designed to

take most conveniently 1 cc. of solution, but satisfactory determinations can be made with smaller and larger amounts. With the mercury bulb at position 2, and cock *f* in the position shown in the figure, the solution is admitted from the cup into the 50 cc. chamber, leaving just enough above the cock to fill the capillary *b*. The cup is washed twice into the pipette with about 0.5 cc. of water each time, and finally 0.5 cc. of 5 per cent sulfuric acid is run in. In plasma analyses a small drop of caprylic alcohol to prevent foaming should precede the sulfuric acid (see "Remarks on details" below).

It is not necessary that exactly 1 cc. of wash water and 0.5 cc. of acid shall be taken, but the total volume of the water solution introduced must extend exactly to the 2.5 cc. mark on the apparatus, if the special formulas of Table I are to be used. As each portion of water is added, enough is left above the cock to fill the capillary, so that no air can enter the latter with the next solution that is added. After the acid has been admitted a drop of mercury is placed in *b* and allowed to run down the capillary as far as the cock in order to seal the latter. Whatever excess of the sulfuric acid remains in the cup is washed out with a little water.

After all the solutions are in the pipette, the upper cock being closed and sealed with mercury, the mercury bulb is lowered and hung at position 3, and the mercury in the pipette is allowed to run down to the 50 cc. mark, producing a Torricellian vacuum in the apparatus. When the mercury (not the water) meniscus has fallen to the 50 cc. mark, the lower cock is closed, and the pipette is removed from the clamp. Equilibrium of the CO_2 between the 2.5 cc. of water solution and the 47.5 cc. of free space in the apparatus is obtained by turning the pipette upside down fifteen or more times, thus thoroughly agitating its contents. The pipette is then replaced in the clamp.

By turning the lower cock the water solution is now allowed to flow from the pipette completely into *d* without, however, allowing any of the gas to follow it. The levelling bulb is then raised in the left hand, while with the right the cock is turned so as to connect the pipette with *c*. The mercury flowing in from *c* fills the body of the pipette, and as much of the calibrated stem at the top as is not occupied by the gas extracted from the solution. A few hundredths

of a cc. of water which could not be completely drained into *d* float on top of the mercury in the pipette, but the error caused by reabsorption of carbon dioxide into this small volume of water is negligible

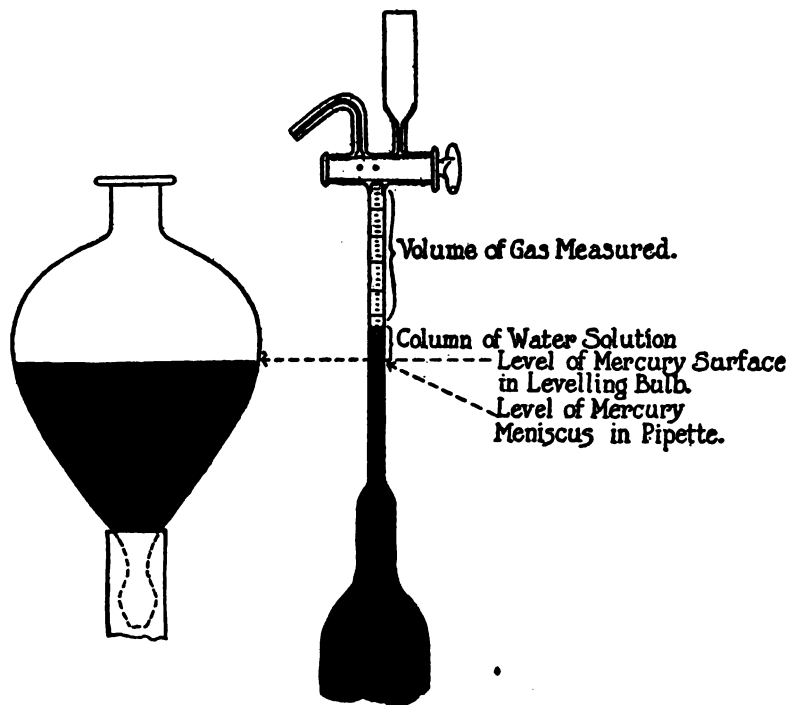


FIG. 2.

if the reading is made at once. The mercury bulb is placed at such a level that the gas in the pipette is under atmospheric pressure, and the volume of the gas is read on the scale.⁶ This concludes the

⁶ In order to have the column in the pipette exactly balanced by that outside, the surface of the mercury in the levelling bulb should be raised until it is level with the mercury meniscus in the pipette, and then, for entire accuracy, raised above the latter meniscus by a distance equal to $\frac{1}{4}$ the height of the column of water above the mercury in the pipette (Fig. 2). As the water column is, as a rule, only about 10 mm. high, the correction that has to be estimated is less than 1 mm. of mercury, *i.e.*, the entire correction for the water column, is not enough to influence results appreciably.

analysis as it is ordinarily done, and the results are calculated with Table I, or, when plasma CO_2 capacity is being determined, with the table on p. 344 of the preceding paper.

After the determination has been finished, the levelling bulb is again lowered without opening the upper cock, and most of the mercury is withdrawn from the pipette through *c*. The water solution from *d* is readmitted and, the levelling bulb being raised to position 1, the water solution, with a little mercury, is forced out of the apparatus through *a*.⁶

The apparatus is now ready for another determination. It is not necessary to wash it out, since the few drops of water which remain in it attached to the walls hold no measurable amount of carbon dioxide. One can, consequently, perform series of determinations at the rate of one every 3 or 4 minutes.

When not in use the entire apparatus should be filled with water. Aside from keeping the cocks properly greased, this is about the only special attention it requires. The mercury is occasionally cleaned by straining it through chamois skin.

Remarks on Details of the Determination.

As a precaution it is advisable immediately before starting the analysis to wash the cup of *b* with a carbonate-free solution of about 1 per cent concentration of ammonia. This treatment assures an alkaline reaction in the cup, which is essential to prevent the possible escape of carbon dioxide from the solution analyzed. The precaution is particularly advisable because the last fluid regularly passing through *b* in each determination is 5 per cent sulfuric acid. Ordinary ammonia solution can be made carbonate-free by adding a small amount of saturated barium hydrate solution. The barium carbonate is filtered off, and the excess of barium remaining is precipitated with a little ammonium sulfate.

An ordinary 0.5 or 1.0 cc. Ostwald pipette may usually be employed in transferring the solution to be analyzed to the receiving cup *b*.

⁶ It is well to have a funnel draining into a special vessel to catch the water residues and mercury overflow from *a*. A considerable amount of mercury is thus regained if many analyses are run. It requires only straining through cloth or chamois skin to prepare it for use again.

The pipettes may be calibrated either for delivery to a mark on the lower stem, or for blowout delivery. In the latter case the final drop is expressed by closing the top of the pipette with the forefinger of one hand, and warming the bulb with the palm of the other. The expansion of the air in the bulb of the pipette forces the drop out at the tip, without following it with a stream of air. The blowout pipette may be used for any solution in which the CO_2 tension is less than one-fifth of an atmosphere. If it is higher, the pipette calibrated to deliver between two marks must be used, as an appreciable amount of CO_2 escapes from the upper layer of solution in the pipette. In the doubly marked instrument this layer is not expelled, and does not influence the analysis.

If the solution analyzed is, like blood plasma, viscous and likely to foam when the gas leaves it, it is convenient, though not absolutely necessary, to add a small drop of caprylic alcohol. With plasma, 0.02 cc. of the alcohol is sufficient to prevent foaming, and does not affect the results. It is measured most conveniently from a burette made by fusing a capillary stop-cock onto a pipette graduated into 0.01 cc. divisions. The drop of caprylic alcohol is placed in *b* before the 0.5 cc. of acid is added, and is permitted to flow entirely into the capillary above *e*. It is then pushed into the pipette ahead of the acid when the latter is admitted. If the alcohol were not trapped in the capillary, it would float on top of the acidified water solution next added and be impossible to transfer to the chamber below.

It is desirable to keep the amount of caprylic alcohol small, as larger amounts may appreciably increase the results, because of the vapor tension of impurities which the alcohol may contain, and because it dissolves much more air per unit volume than does water. Every lot of caprylic alcohol used should be tested by analyzing standard carbonate solutions with and without the addition of the alcohol. If the latter causes an appreciable error it should be redistilled, preferably under reduced pressure. We have always found Kahlbaum's caprylic alcohol, "*Oktylalkohol, Sekundär I*" to give satisfactory results without purification, but this is not the case with all brands. Satisfactory caprylic alcohol can be made by letting castor oil stand over night with an equal volume of concentrated sodium hydrate solution, and distilling from an oil bath (Beilstein, i, 238).

Direct Determination of the Dissolved Air and of the Carbon Dioxide Not Removed by the First Extraction.

The gas obtained by a single extraction represents, according to the temperature, from 95 to 96 per cent of the carbon dioxide that was in the solution analyzed, plus all of the air, 0.04 to 0.05 cc., dissolved under atmospheric pressure by the 2.5 cc. of water introduced into the pipette. This volume of air, which must be deducted from the total gas volume, we usually calculate from the solubility of air in water at the room temperature prevalent (the solutions being shaken with air before use, in order to make certain that they are saturated). The air can, however, readily be determined after absorption of the carbon dioxide. For the direct determination, one admits through the upper cock, after measuring the gas, a little 10 per cent potassium hydrate solution, which in running down the inner wall of the calibrated tube absorbs all the carbon dioxide. The mercury bulb is then held at the proper level⁶ and the volume of residual air is read off on the upper part of the scale.

As stated above, from 4 to 5 per cent of the carbon dioxide remains in solution in the water after equilibrium has been reached. This is because the Henry coefficient $\frac{\text{CO}_2 \text{ per cc. atmosphere}}{\text{CO}_2 \text{ per cc. water}}$ varies between 1 and 0.8 over the ordinary range of room temperature. In order to determine this unextracted carbon dioxide directly, the gas and water in the top of the pipette are, after the gas volume is read, forced out through *a*. In case alkali has been run in for a direct determination of the air, a little acid is run in to wash out the pipette and is then removed through *a*. The pipette is then evacuated, the mercury run down to the 50 cc. mark, and the water solution in *d* readmitted into the 50 cc. chamber. The apparatus is then shaken as before by inverting it ten or twelve times, the water solution is drawn off into *d*, and the extracted gas measured in the calibrated upper stem of the pipette. The second extraction removes 95 per cent of the 4 or 5 per cent of the total carbon dioxide which was not taken out by the first extraction. Consequently the two extractions give 99.8 per cent of the carbon dioxide that was dissolved in the solution analyzed.⁷

⁷ No measurable amount of air is obtained by the second extraction. The solubility of air is so slight that it is all removed by the first extraction.

Calculation of Results.

When the determination is direct throughout, the carbon dioxide being completely obtained by two extractions, while the admixed air in the first gas extract is measured after absorption of the carbon dioxide, one has merely to multiply the total volume of CO_2 obtained by the weight of 1 cc. of moist CO_2 at the prevailing temperature and barometric pressure.

The second extraction can, however, like the determination of admixed air in the first gaseous extract, be dispensed with by utilizing Henry's law. The volume of water and of the free space in the evacuated pipette being fixed, the proportion of carbon dioxide remaining in the water when equilibrium has been established can be accurately calculated from the solubility coefficient in a manner which will be discussed below. By utilization of this principle the entire determination can be reduced to one extraction and the measurement of the gas extracted, the correction for unextracted carbon dioxide, as well as that for the air carried into the system in water solution, being made by calculation, with results as accurate as can be obtained when both corrections are determined by direct measurement. In fact, the calculation of these two corrections by Henry's law is as a rule somewhat more accurate than their direct determination, as the solubility coefficients for air and carbon dioxide are accurately known, and the experimental error involved in the two extra readings is dispensed with when the corrections are calculated from the solubilities.

The formula for the calculation is developed as follows.

$V_{0^\circ, 760}$ = Volume of carbon dioxide, reduced to 0° , 760 mm. in solution analyzed.

V = Volume of gas, obtained by one extraction and measured at atmospheric conditions of t° temperature and B mm. barometric pressure.

f = temperature factor for reduction of volume of gas, measured moist at t° , to volume occupied by dry gas at 0° .

S = cc. of water solution introduced into apparatus.

α_{air} = solubility coefficient of air in water.

α_{CO_2} = " " " CO_2 " "

The solubility coefficients α_{air} and α_{CO_2} used here express the cc. of air or CO_2 measured at t° (not 0°) dissolved by 1 cc. of water in contact with pure air or CO_2 .

Since S cc. of water, saturated with air at atmospheric pressure and therefore containing $S \alpha_{\text{air}}$ cc. of air, is introduced into the apparatus, and is completely removed by the first extraction, the volume of CO_2 in the CO_2 + air mixture measured after a single extraction is $V - S \alpha_{\text{air}}$.

Since, however, $\frac{S}{50}$ of the vacuum chamber is occupied by water at the time of the extraction, $\frac{S}{50} \alpha_{\text{CO}_2}$ parts of the carbon dioxide present remain dissolved in the water phase. The volume of CO_2 extracted is therefore only $1 - \frac{S}{50} \alpha_{\text{CO}_2}$ of the total, and must be divided by this value to give the total. Therefore:

$$\text{Total CO}_2 \text{ measured at room temperature} = \frac{V - S \alpha_{\text{air}}}{1 - \frac{S}{50} \alpha_{\text{CO}_2}}$$

When, as in the routine analyses, $S = 2.5$ cc. the equation becomes

$$\text{Total CO}_2 \text{ measured at room temperature} = \frac{V - 2.5 \alpha_{\text{air}}}{1 - 0.05 \alpha_{\text{CO}_2}}$$

Introducing the corrections for temperature and barometer, we have

$$(1) \quad V_{0^\circ, 760} = (V - 2.5 \alpha_{\text{air}}) \times \frac{f}{1 - 0.05 \alpha_{\text{CO}_2}} \times \frac{B}{760}$$

The values of f , α_{CO_2} , and α_{air} with temperature coefficients sufficiently exact to affect results calculated with the above formula by not more than 0.1 per cent between 15° and 30° are⁸

$$f = 0.999 - 0.0046 t$$

$$\alpha_{\text{CO}_2} = 1.412 - 0.0225 t$$

$$\alpha_{\text{air}} = 0.0255 - 0.00033 t$$

Introducing these values, we have

$$V - 2.5 \alpha_{\text{air}} = V - 0.063 + 0.0008 t$$

$$\frac{f}{1 - 0.05 \alpha_{\text{CO}_2}} = 1.074 + 0.0061 t + 0.000,005,5 t^2$$

$$= 1.074 + 0.0059 t \text{ (between } 15^\circ \text{ and } 30^\circ \text{)}$$

⁸ The values for α_{CO_2} and α_{air} are those of Bohr and Bock (*Chem. Kalendar*, 1912, i, 271 and 275). They are recalculated so that the values are here expressed in volumes of CO_2 at t° , 760 mm., instead of 0° , 760 mm., as in the original.

Whence:

$$(2) V_{0^{\circ}}, \quad (V - 0.063 + 0.0008 t) (1.074 - 0.0059 t) \frac{B}{760}$$

To express the results in mg. of carbon dioxide, we multiply the second factor by 1.964, the weight of 1 cc. of the gas at 0° , 760 mm.

We then have:

$$(3) \text{Mg. CO}_2 = (V - 0.063 + 0.0008 t) (2.109 - 0.0116 t) \frac{B}{760}$$

For convenience in calculating results obtained at different temperatures the following table is given. If, for example, 0.69 cc. of gas are measured at 22° , 750 mm., the mg. of CO_2 indicated = $(0.690 - 0.045) \times 1.854 \times \frac{750}{760} = 1.180$ mg.

If the result is desired in cc. of CO_2 reduced to 0° , 760, it is $(0.690 - 0.045) \times 0.944 \times \frac{750}{760} = 0.601$ cc.

The degree of accuracy attainable by this method is indicated by the following analyses. The solutions were made by weight from Merck's reagent anhydrous Na_2CO_3 , which had been heated in an oven to insure its dryness. 1 cc. of solution was used for each analysis. The second extraction of carbon dioxide was performed in each case, and the volume of carbon dioxide obtained by it is given in the last column. The next to the last column gives the volume of carbon dioxide allowed for by Equation 1 as the residual volume of CO_2 gas not removed from solution by the first extraction; *i.e.*, the difference between the values calculated by Equation 1 as it stands, and the values calculated without the term $-0.05\alpha_{\text{CO}_2}$ in the denominator.

Comparison of the last two columns shows that the calculated and observed amounts agree within the limit of error of the latter.

The results show both that the method is free from serious error, and that the extra labor of determining the "dissolved air" to be subtracted, and the "unextracted carbon dioxide" to be added to the first reading, can be dispensed with; as these corrections can be calculated with probably even greater accuracy than they can be determined in this apparatus. Consequently the necessary labor is reduced to extracting the carbon dioxide once from its solution, reading the volume of gas obtained, and calculating the result with the aid of Table I. The results of blood plasma analyses are given in accompanying papers.

TABLE I.

Carbon Dioxide Indicated by Reading of V Cc. of Gas after a Single Extraction.

Temperature of analysis.	Air dissolved in 2.5 cc. H ₂ O.* Subtract this from V and multiply result by A to calculate mg. CO ₂ , by C to calculate cc. CO ₂ reduced to 0°, 760 mm.	A.	C.
°C.	cc.	mg. $\frac{B^{**}}{760} \times 1.935$	cc. $\frac{B^{**}}{760} \times 0.985$
15	0.051	" 1.924	" 0.980
16	0.050	" 1.912	" 0.974
17	0.049	" 1.900	" 0.968
18	0.048	" 1.889	" 0.962
19	0.048	" 1.877	" 0.956
20	0.047	" 1.866	" 0.950
21	0.046	" 1.854	" 0.944
22	0.045	" 1.842	" 0.938
23	0.045	" 1.831	" 0.932
24	0.044	" 1.819	" 0.927
25	0.043	" 1.808	" 0.921
26	0.042	" 1.796	" 0.915
27	0.041	" 1.784	" 0.909
28	0.040	" 1.773	" 0.903
29	0.040	" 1.761	" 0.897
30	0.039		

* This correction can be used when, as in the case of plasma and most other solutions, the oxygen and nitrogen dissolved by water from air at atmospheric pressure are the only gases besides carbon dioxide given off in appreciable amounts by the acidified solution. In the analysis of whole blood, however, one also extracts part of the oxygen bound by the hemoglobin, and in this case the volume of gases other than CO₂ must be determined directly after absorbing the CO₂ with 10 per cent KOH solution.

** For convenience the values of $\frac{B}{760} \left(= \frac{\text{barometric pressure in mm.}}{760} \right)$ for ordinary atmospheric pressures are given after Table I at the end of the paper immediately preceding this.

TABLE II.
Analyses of Standard Carbonate Solutions.

Na ₂ CO ₃ per liter solution.	Volume of gas observed in analysis of 1 cc. solution. First extraction.	Temperature.	Barometer.	CO ₂ per cc. solution. Calculated by Table I.	CO ₂ per cc. solution present.	Unextracted CO ₂ .	
						Allowed for in calculation.	Obtained by second extraction.
gm.	cc.	°C.	mm.	mg.	mg.	cc.	cc.
3.616	0.862	24	758	1.491	1.500	0.037	0.035
"	0.866	24.5	758	1.501	"	0.036	0.033
"	0.868	25	758	1.504	"	0.036	0.035
"	0.867	25	758	1.502	"	0.036	0.034
2.410	0.595	24	756	1.004	1.000	0.024	0.022
"	0.597	24.5	756	1.006	"	0.024	0.024
"	0.592	23.5	756	1.000	"	0.024	0.024
"	0.594	23.5	756	1.003	"	0.024	0.026
1.205	0.318	22	756	0.501	0.500	0.012	0.010-0.015*
"	0.318	22	756	0.501	"	0.012	"
"	0.316	22	756	0.498	"	0.012	"
"	0.315	22	756	0.497	"	0.012	"

* Entirely accurate measurement was impossible so near the zero point of the scale.

Analysis of Solutions, Such as Whole Blood, Which Yield Other Gases, in Addition to Carbon Dioxide and the Amount of Air Dissolved by Water at Atmospheric Pressure.

With such solutions the carbon dioxide must be determined directly by absorption with potassium hydrate solution, as described on p. 384. The carbon dioxide volume thus obtained is multiplied by the value of A or C in Table I corresponding to the temperature of the analysis.

This method must be utilized when the carbon dioxide content of whole blood is determined, as about two-thirds of the oxygen combined with the hemoglobin is obtained by the extraction of the acid solution.

Micro-Apparatus for Estimation of Carbon Dioxide in Small Volumes of Solution.

The micro-apparatus described below is designed to measure within 1 volume per cent the small amounts of carbon dioxide (0.1 to 0.15 cc.) in 0.2 cc. of blood plasma. As will be seen, the principle of extraction in a Torricellian vacuum is utilized, as with the larger apparatus, but the manner of application is somewhat different from that followed with the latter. Instead of measuring the gas in the extraction chamber after removal of the water, the gas is transferred to a separate measuring chamber without release of the vacuum.

Apparatus.—The nature of the apparatus is evident from the figure. The use of a glass tube instead of rubber as part of the connection between the apparatus and the levelling bulb is necessary because the minute amounts of gas which rubber may give off are sufficient to affect results appreciably. The curve in the lower part of this glass tube serves as a trap to catch any small gas bubbles which might come from the rubber tube forming the remainder of the connection with the levelling bulb. The reasons for other differences in structure become evident when the directions below for using the apparatus are read.

The capillary tube in which the gases are measured is divided into 100 divisions of 0.002 cc. each, numbered from 1 to 100 rather than in absolute volumes, so that when 0.2 cc. of carbonate solution is analyzed each division indicates a volume equal to 1 per cent that of the solution. The different parts of the micro-apparatus are in the same relative proportions as the corresponding parts of the larger apparatus, being one-fifth as large. Consequently when 0.5 cc. of total water solution is introduced the same formula (Table I) can be used for calculating results as in the case of the large apparatus, each division of 0.002 cc. on the smaller corresponding to 0.01 cc. on the larger.

Determination.—The apparatus being entirely filled with mercury, the solution or plasma, usually 0.2 cc., is measured into cup *a* at the top of the apparatus, the tip of the pipette being kept in contact with the liquid in the cup during the delivery. An Ostwald pipette with a heavy walled capillary stem of about 1 mm. inner diameter is

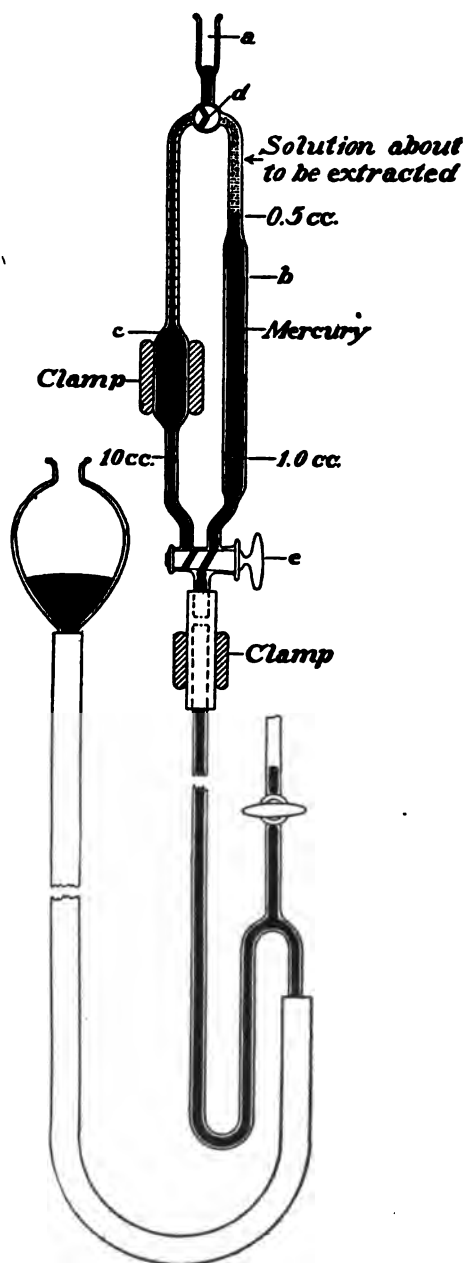


FIG. 3. Micro-apparatus.

used. The pipette is calibrated to deliver 0.2 cc. between two marks, the lower of which is 3 to 4 cm. above the tip, as with such small amounts more accurate results are obtained by draining the pipette between two points than by blowout delivery. The solution is washed from *a* into *b* with two portions of about 0.1 cc. of water each, the water being distributed about the lower part of the wall of *a* with a fine-pointed medicine dropper. Enough 5 per cent sulfuric acid is then admitted to fill *b* down to the 0.5 cc. mark. No caprylic alcohol need be added, even with plasma, to prevent foaming. The admission of the successive portions of liquid from *a* into *b* is best controlled by leaving open the connection between *a* and *b*, and governing the inflow with cock *e*.

The 0.5 cc. of water solution being all within *b*, cock *d* is turned to connect *a* with *c*, and a little mercury is forced up into *a* where it serves to provide the necessary mercury seal to cock *d*.

Cock *d* is now turned to the position shown in the drawing, which represents the apparatus at this stage of the determination. Chamber *b* is evacuated till the mercury has fallen to the 10 cc. mark. Cock *e* is then closed, the clamp about *c* is loosened, and the apparatus is shaken by moving it to the horizontal position and back a dozen times. Extraction seems even quicker than with the larger apparatus. The clamp below *e* is not loosened during this or any other part of the analysis.

The apparatus is returned to the upright position, and *c*, which has hitherto been full of mercury, is evacuated. Then, the levelling bulb being raised to about the level of *e*, *d* is turned to connect *b* and *c*, and mercury is at once admitted through *e* into *b*. As *b* fills with mercury the rarefied gases in it pass over into *c*. When the solution in *b* has risen to the narrowed upper portion of the chamber the rate of flow is retarded, and is cautiously allowed to progress until the meniscus of the water just or almost reaches cock *d*. Cock *e* is then closed and *d* is turned to connect *a* and *b*. Mercury from *a* flows into the evacuated bore of *d* and seals the cock with a completeness which is necessary for an accurate final reading of the gas now in *c*. The amount of gas trapped in the bore of *d* is negligible.

Cock *e* is now turned to admit mercury into *c*, and the volume of gas trapped in the calibrated capillary at the top of *c* is read off at

atmospheric pressure, the levelling bulb being so held that the surface of the mercury in it is level with the mercury meniscus in the capillary. The results are calculated by the same tables used for the larger apparatus. The barometric pressure must be corrected, however, for the effect of capillary attraction on the mercury in the calibrated capillary. This correction is readily determined by connecting *c* with the outer atmosphere through *d*, holding the levelling bulb near to the calibrated capillary, and measuring the difference between the levels of the mercury surfaces in the bulb and the capillary respectively. In our apparatus, with a capillary of somewhat more than 1 mm. bore, the capillary action held the mercury meniscus 4 mm. below the mercury surface in the levelling bulb. Consequently 4 mm. is deducted from barometric readings in making the gas calculations.

Before every analysis, even of a series made at one time, the apparatus must be cleared of any minute bubbles by lowering the mercury bulb and evacuating both chambers *b* and *c* as well as the tube for some distance below cock *e*. When the mercury is readmitted a little is forced up into *a* from both chambers, carrying with it any bubbles which may have been detached from the walls. This precaution is absolutely necessary if results are to be obtained within the 1 per cent limit of accuracy.

Air is admitted into the apparatus as seldom as possible because it is adsorbed by the glass walls and held, even after readmission of mercury, in amounts sufficient to cause gross errors. In case air is admitted, it must be removed by evacuating the apparatus twice in the manner described in the preceding paragraph before an analysis is performed.

Calibration of the Micro-Apparatus.

The capillary measuring tube may be calibrated in two ways, either by weighing the mercury which it delivers, or by analyzing standard solutions of Na_2CO_3 and ascertaining the difference between the observed volumes and those calculated with the aid of Table I.

In the mercury calibration it is essential that the capillary should be *wet*, because, from condensed water vapor, it is always wet when analyses are performed. Consequently a drop of water is run down

the calibrated capillary and then expelled by mercury. If the tube is clean, just enough water will remain attached to the walls to form a layer over the mercury so thin that the menisci of mercury and water meet in the middle of the capillary. This is the condition under which readings of analyses are made.

TABLE III.

NaCO ₃ per liter of solution.	Temperature.	Barometer.	Gas reading observed. Scale divisions of 0.002 cc.	Gas reading calculated. Scale divisions.	Scale correction.	Average correction.
gm.	°C.	mm.				
3.616	23	748	86.0	87.1	+1.1	+1.2
	"	"	85.9	"	+1.2	
	"	"	85.9	"	+1.2	
	"	"	85.9	"	+1.2	
1.446	"	"	38.6	37.8	-0.8	-0.6
	"	"	38.3	"	-0.5	
	"	"	38.3	"	-0.5	
0.723	"	"	22.1	21.0	-1.1	-1.1
	"	"	22.0	"	-1.0	
	"	"	22.2	"	-1.2	
	"	"	22.1	"	-1.1	

For delivery of the mercury, a glass tube drawn out into a very fine capillary is attached to the tube below cock *e*. The delivery of the mercury is controlled with cock *e*, and the tip of the capillary is touched to the mercury in the weighing bottle after the delivery of each portion.

For calibration with standard carbonate solutions one simply makes analyses in the usual manner.

Fig. 4 indicates the error that would be introduced by calibrating the apparatus with dry walls; it also shows how closely the results of calibrating by means of standard carbonate agree with those by the mercury method when the mercury is delivered from the moist capillary.

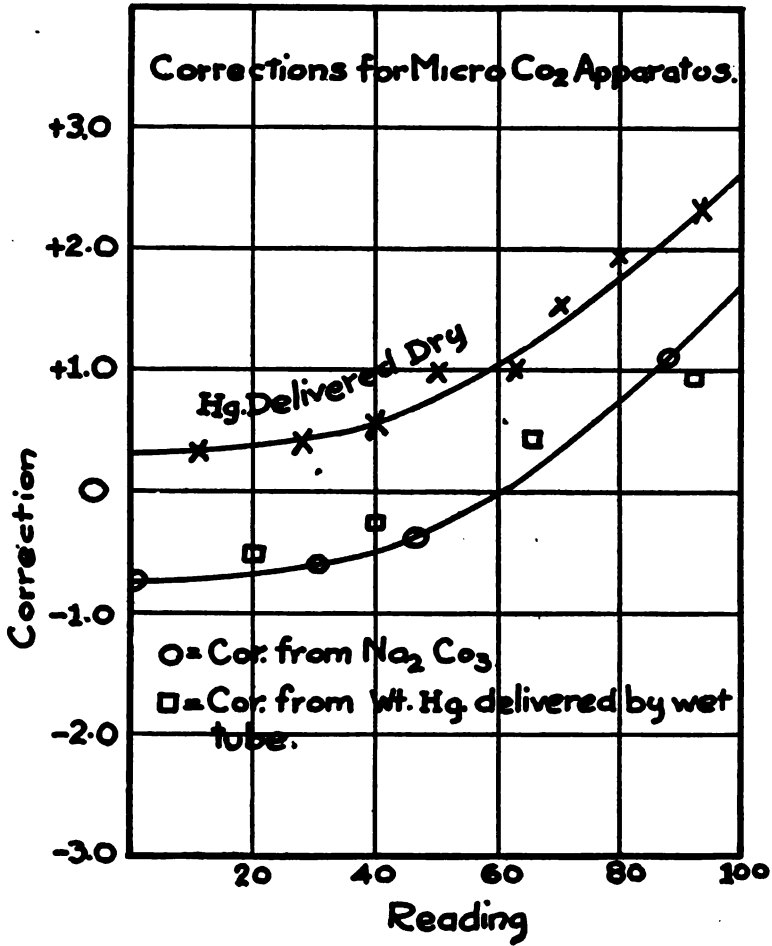


FIG. 4.

SUMMARY.

A simple one-piece apparatus is described for determination of the carbon dioxide or carbonate content of water solutions. It has been designed especially for analysis of 1 cc. samples of blood plasma, but is applicable to water solutions in general as well as to the determination of dissolved gases other than carbon dioxide.

The entire analysis is performed at room temperature, requires about 3 minutes, and without especial precautions is capable of accuracy to within 1 per cent of the amount determined.

A micro-apparatus designed on a similar principle is described. With it the carbon dioxide content of 0.2 cc. of plasma can be determined with an accuracy of 1 volume per cent.

STUDIES OF ACIDOSIS.

III. THE ELECTROMETRIC TITRATION OF PLASMA AS A MEASURE OF ITS ALKALINE RESERVE.

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It has been definitely established that the reaction of the blood is, under normal conditions, constant (Lundsgaard, 1912) and may be kept constant even under pathological conditions involving great lowering of the alkaline reserve (Michaelis, p. 105; Benedict, 1906; Peabody, 1914; for more detailed discussion see Paper I of this series). Consequently, the hydrogen ion concentration (C_H) of the blood as drawn from the veins cannot be taken as an indicator of the alkaline reserve.

We may expect, however, that when a given amount of acid is added to the blood, the resultant change in C_H will be greater the less the reserve alkali; or conversely, that less acid will be necessary to produce a given increase in C_H . The latter principle is essentially that used in methods for titration of the blood with indicators, acid being added until the C_H is reached at which the indicator changes color; and titration methods have contributed greatly to our knowledge concerning the alkaline reserve of the blood and its changes in acidosis (Jaksch, Magnus-Levy, Pembrey, and Spriggs). The interpretation of such results, however, is clouded by two deficiencies inherent in the method. First, the indicators (Bjerrum, 1915), such as methyl orange, which can be used to titrate carbonates as alkali change color at such a high C_H that the titration measures, in addition to the bicarbonate, also an acid binding power of the proteins quite out of proportion to the amounts of acid which these substances can bind within the limits of C_H possible in life (the phosphates of whole blood also act as buffers like proteins, but they

play a minimal rôle in plasma). Second, the proteins diminish by their buffer effect and by absorption of indicator the sharpness of the end-points, and thereby decrease the accuracy of the titrations. Attempts to overcome these difficulties by precipitating the proteins with neutral agents such as ammonium sulfate are criticized because the precipitated protein carries down with it a considerable part of the alkali (Hoppe-Seyler). Of the above two difficulties the first is inherent in every mode of titrating blood against strong acids. The second, however, can be avoided by determining the C_H of the end-point electrometrically instead of by indicators, so that even with small amounts of blood or plasma results reproducible with a high degree of accuracy can be obtained. The present paper presents the results of an attempt to develop the electrometric titration into a form practicable for use with normal and pathological plasmas,—and the comparison of the method so developed with the determination of the carbon dioxide combining capacity of the plasma (Van Slyke and Cullen, 1917), and of the carbon dioxide tension of the alveolar air (see Paper VI of this series).

The principles of the gas chain method and its practice have been so thoroughly reviewed in recent literature (Soerensen, 1912; Hildebrand, 1913; Michaelis, 1914; Clark and Lubs, 1916) that only the necessary details are given here.

Apparatus.

All determinations of the H^+ concentration were made by use of the well known gas chain.



The mercury for the calomel cells was purified in the wet way, distilled three times by Hulett's method (1911), and filtered. The calomel was made from this mercury by the method of Loomis and Acree (1911).¹ The $N/10$ KCl solution was prepared by weight

¹ Electrodes prepared from this calomel have not been very satisfactory. In later work with the gas chain the calomel has been prepared electrolytically as described by Ellis (1916). The calomel has been kept under acid and prepared for use by washing by decantation only. Cells prepared from such calomel remain entirely constant for long periods.

from recrystallized Kahlbaum's KCl and the standardization verified by chloride determinations by the McLean and Van Slyke method.

The hydrogen was taken from tanks of the Standard Oxygen Company and was washed through solutions of HgCl_2 , KMnO_4 , pyrogallol (twice), dilute H_2SO_4 , and water. This proved to be a convenient and satisfactory source of hydrogen.

The electrodes were made from No. 16 and No. 18 platinum wire sealed into glass tubes and platinized with platinum black. Small Clark electrode vessels of about 2 cc. capacity were used, together with the ingenious Clark shaking device (Clark, 1915). This vessel is similar in principle to that of Hasselbalch (1911, 1913), but is superior to it both practically and theoretically. It is designed to give maximum surface of solution and elimination of dead space, and to reduce the contact potential between the solution and saturated KCl solution to a negligible quantity. It is easily manipulated, requires only a small amount of fluid, and gives such thorough contact of solution and electrode that equilibrium is established inside of a few minutes. This apparatus is especially convenient when working with carbon dioxide containing fluids, for it allows easy and economical renewal of the solution without change of hydrogen, the procedure proposed by Hasselbalch. The substitution of a 60° 3-way stop-cock with a 2-way key for the lower stop-cock of the Clark vessel is a decided convenience.

Several calomel electrodes were kept on hand, and compared frequently with one another. An absolute standard was thus maintained, and the readings corrected with it. Weston cells calibrated by the Bureau of Standards gave the standard potential. The readings were taken during the earlier part of the work with a bridge of 1,110 ohms resistance boxes and electrometer as zero point instrument. The external resistance was so adjusted that ohms equalled millivolts. Later a Leeds and Northrup potentiometer with a galvanometer as zero point instrument was installed.

The determinations were run at room temperature, $18-24^\circ$, and temperature corrections applied. The accuracy of the entire determination was tested often by means of Soerensen's standard phosphate and Walpole's standard acetate mixtures of known hydrogen ion concentration.

The N/50 acid used in the titrations was prepared by weight from a standard HCl prepared by Hulett and Bonner's (1909) method and checked by gravimetric silver chloride determinations.

Hasselbalch and Gammeltoft (1915) report that freshly platinized electrodes are essential. Our experience entirely corroborates this observation. The electrodes were always carefully cleaned before each determination in accordance with the following routine. The washed electrodes were placed as cathode in the reducing vessel containing 5 per cent H_2SO_4 for 3 minutes where the bubbling hydrogen removed a considerable quantity of precipitated protein that could not be washed off. Then they were rinsed with water, placed as cathode in the platinic chloride solution for 2 minutes, rinsed, returned to the reducing vessel for 3 minutes, rinsed thoroughly with tap water, and finally with distilled water. The electrodes were often checked against the standard solutions.

Calculation of Results.

All H^+ concentrations are expressed by Soerensen's symbol pH, the negative Briggs logarithm of the hydrogen ion concentration. pH values are more easily visualized and plotted than those of C_{H} , the actual hydrogen ion concentration. The equation for the calculation of results was:

$$\log \frac{1}{C_{\text{H}}} = \text{pH} = \frac{E - 0.337}{0.0577 [1 + (t^\circ - 18^\circ) 0.0002]}$$

In our work the variations in potential and temperature were such that it was most convenient to construct tables giving pH directly from the observed readings. We used the rounded value of 0.337 for the N/10 calomel electrode adopted by the Potential Commission (Auerbach).

Preparation of Solution.

All our determinations have been made on plasma rather than whole or defibrinated blood; because removal of the corpuscles eliminates the greater part of the oxygen and consequently allows much quicker reduction of the solution by the hydrogen electrode.

The blood was drawn into a tube containing a small amount of powdered potassium oxalate (always less than 0.5 per cent) to prevent clotting, centrifuged at once, and the plasma pipetted off. Experiments proved that the oxalate had no effect on the determination. If it was impossible to run the determination at once, the plasma was placed in a stoppered paraffined tube in the refrigerator.

In comparing the alkaline reserve of different plasmas by titration with acid, two methods are available. First, the amount of acid required to bring the plasma to a definite H^+ concentration may be determined by a series of determinations and consequent plotting of the titration curve. Or, second, the H^+ concentration resulting from the addition of an arbitrarily fixed amount of acid may be found. This is the most economical, both of time and blood, the deciding considerations when series of observations on a number of patients are desired. We therefore used the former only to select suitable conditions to use in this investigation.

However, since the initial reaction of all plasmas is practically constant and the titration curve is almost a straight line, it is entirely possible to construct the curve from one determination and from it determine the amount of acid required to bring the plasma to any desired reaction.

It is evident from the analysis of the equilibrium $\frac{H_2CO_3}{NaHCO_3}$ that all samples must be brought to a definite CO_2 content. The conditions under which the blood is drawn, the time elapsing before determination, etc., are so variable that the plasma cannot be used as it is. Its CO_2 content must either be fixed under definite conditions, or the CO_2 must be removed.

Two procedures have been followed. The first was worked out for and used throughout the investigation of the first series of experiments on diabetic acidosis; the second was developed on the basis of experience with that series.

Technique of Determination.

Procedure A.—It was found that two successive shakings in an evacuated vessel, as described in the next paragraph, reduced the CO_2 content to a fairly constant level. Experiments with $N/50$ HCl

showed that more than three volumes of this acid per volume of plasma precipitated some of the proteins. The addition of one volume of $N/50$ acid to one volume of plasma (Fig. 1) brought normal plasma to about the neutral point. Plasma with less than normal alkaline reserve would, under the same conditions, fall on the acid side of the neutral line.

The oxalated blood was centrifuged and the plasma pipetted off. About 3 cc. of the plasma were placed in a 300 cc. separatory funnel, which was evacuated by means of a water aspirator to a pressure of about 20 mm. The funnel was then rotated for 3 minutes to insure maximum surface and permit the escape of carbon dioxide from the solution. Air was then admitted, the funnel was evacuated, and was again rotated for 3 minutes. Exactly 1 cc. of this plasma was then measured into a 3 cc. dropping funnel, and 1 cc. of $N/50$ HCl added (calibrated 1 cc. pipettes were used for both acid and plasma); the funnel was closed by means of a small rubber stopper and the solution mixed, without shaking. The mixed solution was then allowed to flow through a small piece of rubber tubing into the Clark electrode, displacing part of the hydrogen with which the vessel had been filled. The care taken in mixing and introducing the acidified solution was to prevent loss of CO_2 . The vessel was then shaken for 3 or 4 minutes, the electrode adjusted to minimum contact, and the potential read. The vessel was shaken again 3 or 4 minutes, a fresh contact with the saturated KCl solution was made, another reading taken, and the process was continued until equilibrium was reached, which usually was at the second or third reading.

Procedure B.—The method outlined under A is open to the objection that careful attention is required to bring the carbon dioxide to the arbitrary level. This possibility of error can be eliminated by adding enough acid to complete the reaction $NaHCO_3 + HCl = NaCl + H_2CO_3$, and then removing all the carbon dioxide by evacuation. The carbon dioxide tension of the plasma is thus eliminated as a factor in the results, and the hydrogen ion concentration is dependent on the equilibrium between the plasma buffers, chiefly the proteins, and the excess of acid not neutralized by bicarbonate. It was found that the addition of two volumes of $N/50$ HCl to one of plasma was sufficient to effect complete decomposition of the bicarbonate, and

the resulting solution after removal of the carbon dioxide showed a C_{H^+} of about $10^{-5.6}$ ($\text{pH} = 5.6$). At this point the equilibrium in the electrode vessel is obtained much quicker than at the neutral point. This procedure has, over A, the advantages of greater rapidity, the use of smaller amounts of plasma, and the elimination of variations in the carbon dioxide content as a source of error. It has the disadvantage, compared with procedure A, in that the differences in pH corresponding to given differences in "alkaline reserve" are not so great.

In procedure B two volumes of N/50 HCl are added to one of plasma (the amounts being usually 2 and 1 cc. respectively) in a small separatory funnel, 25 to 100 cc., the solution is exhausted with a water aspirator to about 20 mm., and agitated for 1 minute. One exhaustion completely removes the carbon dioxide. The solution is then transferred to the electrode vessel by a pipette, allowed to displace part of the hydrogen, and the reading obtained in the same manner as in A.

EXPERIMENTAL.

The Determination of the Optimum Amounts of N/50 Acid.

Samples of oxalated plasmas were exhausted twice with the water aspirator and the H^+ of the exhausted plasma determined. Then a series of determinations was made varying the ratio of acid and plasma volume as shown in Fig. 1.

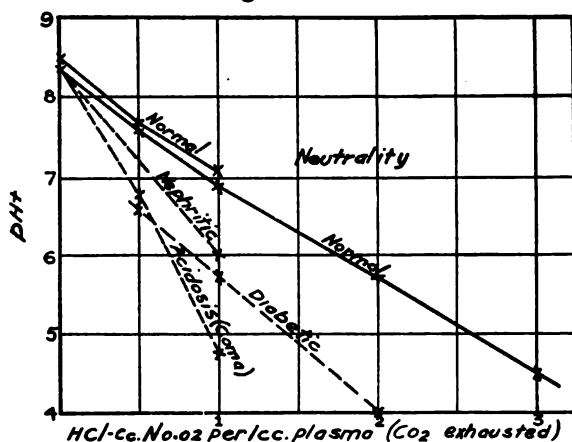


FIG. 1.

It is evident that the addition of one volume of N/50 acid to one volume of plasma brings normal plasma to about the neutral point. The acidosis plasma affords an extreme example of diminished alkaline reserve.

Determination of the Number of Exhaustions Required in Water Aspirator.

Samples of plasma were saturated with alveolar air (5.5 per cent CO₂), portions were placed in separatory funnels, and exhausted on a water aspirator to about 20 mm. for periods of 3 minutes each. During the exhaustion the funnels were rotated constantly to expedite the escape of the CO₂ from the solution. Air was admitted to the funnels between exhaustions. 1 cc. portions were then mixed with 1 cc. portions N/50 HCl in 3 cc. dropping funnels, as described above, and the contents run into the electrode vessels (Table I).

TABLE I.

Number of Exhaustions Needed for Procedure A. pH after Adding One Volume N/50 HCl to Exhausted Plasma.

Human plasma.	Treatment of sample.			
	Saturated with 5.5 per cent CO ₂ .	Exhausted once.*	Exhausted twice.	Exhausted three times.
	pH	pH	pH	pH
1	6.42	6.67	6.69	6.69
2	6.66	6.76	6.78	6.78
3	6.47		6.63	6.63
	6.45		6.62	6.62
4		7.03	7.03	7.03

* Each exhaustion lasted 3 minutes.

It appeared from the above that two exhaustions were sufficient for our purpose, and it was desirable to avoid as far as possible concentrating the plasma by evaporation. Later, however, the perfection of Van Slyke's CO₂ apparatus furnished an accurate and convenient means of checking the above experiments. The amounts of carbon dioxide remaining after successive exhaustions were determined. The plasma was placed in large separatory funnels, the

exhaustions were carried out as described under procedure A, and a 1 cc. sample was removed for analysis after each exhaustion (Table II).

TABLE II.

Number of Exhaustions Needed for Procedure A. Total Residual CO₂ Determined by Van Slyke's Method. Corrected to 0°, 760 Mm.

Human plasma.	Saturated with 6.3 per cent CO ₂ .	CO ₂ remaining after							
		1st 3' exhaustion.	Per cent of CO ₂ at saturation.	2nd 3' exhaustion.	Per cent of CO ₂ at saturation.	3rd 3' exhaustion.	Per cent of CO ₂ at saturation.	4th 3' exhaustion.	Per cent of CO ₂ at saturation.
	cc.	cc.		cc.		cc.		cc.	
G.....	0.79	0.60	76.2	0.54	68	0.49	62	0.48	60
A.....	0.72	0.53	73	0.43	60	0.40	56	0.38	53
An.....	0.75	0.55	73	0.45	60	0.41	54	0.38	51
O.....	0.775	0.57	74	0.52	67	0.46	59	0.43	55

It is evident from Table II that exhaustion was not complete. This was expected. However, the variations after two exhaustions were so small in their effect on pH, as demonstrated in Table I, that accurately reproducible results are obtainable when two exhaustions, carried out as described, are used as the arbitrary standard.

That the arbitrarily selected double exhaustion approximates to a constant carbon dioxide content is also evident from the curves constructed from these values in Fig. 2.

The carbon dioxide as a source of error has been entirely eliminated in procedure B by the use of larger amounts of acid and exhaustion *after* its addition. The following experiment shows this (Table III).

Two volumes of N/50 acid were added to one volume of plasma in a separatory funnel. The funnel was exhausted to about 20 mm. for 1 minute, with constant rotation. 1 cc. was taken for analysis and the exhaustion repeated.

The amounts of residual gas in column 3 are entirely comparable with the "dissolved air" correction in this method; therefore no measurable amounts of carbon dioxide were present.

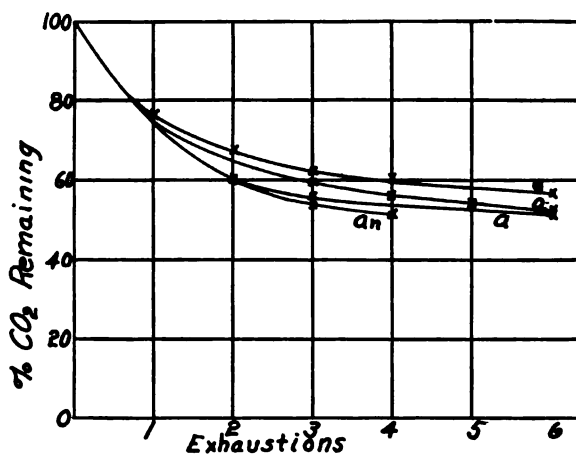


FIG. 2. From Table II.

TABLE III.

Number of Exhaustions in Water Aspirator Needed after Adding Two Volumes of N/50 HCl. Dissolved Gases Determined by Van Slyke's Method. 30° and 762 Mm.

Sample plasma.	Exhausted 3' with shaking. Gas observed.	Dissolved air calculated as present.	CO ₂ present.
	cc.	cc.	cc.
Mixed normal.....	0.04	0.044	0.000
Same saturated with 6.3 per cent CO ₂	0.04	0.044	0.000
A.....	0.045	0.044	0.000
Dog.....	0.04	0.044	0.000
H.....	0.05	0.044	0.000

Comparison of Two Procedures for Removing Carbon Dioxide.

Hydrogen ion concentrations were determined on two plasmas. Each was then divided in two portions, one of which was placed in a separatory funnel and was exhausted 3 minutes as in procedure A. 1 cc. samples were then mixed with various volumes of N/50 acid and the resulting reaction was determined.

To samples of the other portions in separatory funnels varying volumes of N/50 acid were added and the mixtures exhausted for 1

minute. The results are given in Table IV and plotted in Figs. 3 and 4; it is evident that the two series differ but little.

TABLE IV.

Comparison of Procedures A and B.

Plasma.	$\frac{N}{50}$ acid per cc. plasma.	A (exhausted twice before adding acid).	B (exhausted once after adding acid).
	cc.		
P. pH = 8.63	0.5	7.73	7.56
		7.74	7.58
	1.0	6.74	6.68
		6.74	
	2.0	5.43	5.34
		5.42	5.33
M. pH = 8.61	3.0	4.54	4.40
		4.53	4.40
	0.5	7.76	7.83
	1.0	6.88	6.87
	2.0	5.47	5.48

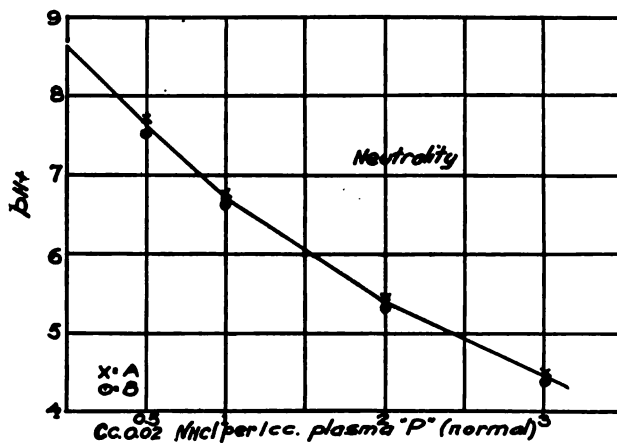


FIG. 3.

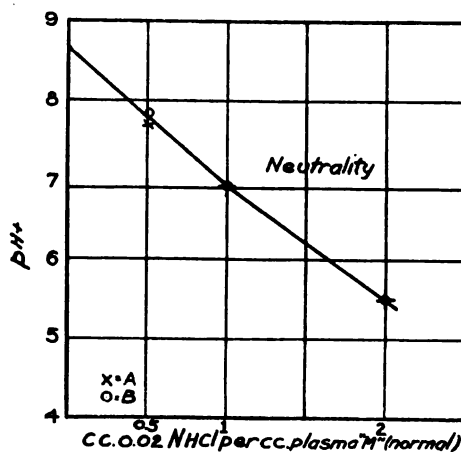


FIG. 4.

Experiment to Determine the Effect of Oxalate on the Titration of Plasma.

Solutions of $m/10$ phosphates and of normal plasma were made up with varying concentrations of potassium oxalate. H^+ concentration determinations were made as indicated in Table V. It is evident that in plasma oxalate up to 0.5 per cent does not produce an appreciable change and that the change produced by 1 per cent is within the allowable limits of experimental error. About 0.2 to 0.3 per cent is ordinarily sufficient to prevent clotting.

TABLE V.

Effect of Oxalate.

On pH of $m/10$ phosphate solution concentration.		On pH of $m/10$ phosphate solution + 1 vol. $N/50$ HCl concentration.		On pH of normal plasma treated by procedure B (2 vol. $N/50$ acid added) concentration.	
K oxalate.	pH.	K oxalate.	pH.	K oxalate.	pH.
<i>per cent</i>		<i>per cent</i>		<i>per cent</i>	
0	7.50	0	6.74	0	5.38
0.2	7.47	0.25	6.74	0.5	5.38
0.6	7.47	0.5	6.74	1.0	5.39
1.0	7.44	1.0	6.72	2.0	5.45
1.6	7.45			4.0	5.53
2.0	7.94				

Effect of Renewing Solutions in Electrodes.

Hasselbalch pointed out the possibility of renewing successively the solutions in the electrode without renewing the H_2 as a means of bringing the CO_2 content of the electrode gas to equilibrium with that of the solution. The following experiment (Table VI) shows that this precaution is unnecessary with the small amount of residual CO_2 present under procedure A.

TABLE VI.

Effect of Renewing Solution without Change of H_2 Atmosphere. New Solutions Were Run into the Electrode Vessel Displacing the Used Solution without Changing the H_2 Bubble.

Plasma.	E.	S.	U.	H.	M.	M. 2.	V.
pH of 1st determination.....	8.13	7.11	6.48	7.03	6.55	6.89	6.99
pH of renewed solution.....	8.14	7.11	6.48	7.03	6.58	6.91	6.99

Accuracy of the Method.

Clark has pointed out the possibility of accuracy attainable with his electrode. With plasma we have considered 1 millivolt = 0.02 pH our permissible error. This amount is very small in comparison with the gross differences we have obtained in the clinical studies.

Table VII gives samples of the results obtained in duplicate determinations.

Titration of Plasma Containing Lactic and β -Oxybutyric Acids.

Since a diminished alkaline reserve is the result of the introduction of abnormal acid products of metabolism, it seemed desirable to investigate the effects of adding such acids to a normal plasma *in vitro*. The results of such experiments are shown in Tables VIII and IX, and Figs. 5 and 6. These experiments were performed as follows:

Experiment I. Curves A and B.—A weighed amount of acid was added to a portion of plasma to make the plasma 0.2 per cent acid solution. This solution was then mixed with untreated plasma in varying proportions and the resulting mixtures were titrated by both A and B methods. The acids were analyzed by adding excess

TABLE VII.

Duplicate Determinations Made with Different Electrodes in Different Vessels.

Procedure A.					Procedure B.				
Plasma (human).	Date.	Temper- ature.	Reading.	pH.	Plasma.	Date.	Temper- ature.	Reading.	pH.
		°C.	milli- volts				°C.	milli- volts	
A.	May 15	23	723	6.57	Dog VIII.		25	630	4.96
		23	722	6.55			23	627.7	4.96
W.	" 25	24	742	6.87	"	June 14	25	627	4.91
Normal.		23.5	740	6.85			23	626.6	4.93
V.	" 26	23	744	6.93	V.* (human).	" 13	25	650.4	5.30
Normal.		23	744	6.93			26	652.5	5.33
							26	651.6	5.31
M.	" 23	24	749	6.99			26	651.6	5.31
Normal.			749.5	7.00			26.5	652.3	5.32
								652.2	5.32
H.	March 21	20	723	6.64	Dog 2 a.	" 10	25	620	4.98
		21	723	6.62			26	622.6	4.91
					" 2 b.	" 10	25	631.5	4.98
							26	632	4.97
					" 3.	" 11	22	630	5.00
								630.1	5.00

* These six determinations were run with six distinct pipettes, funnels, and electrodes, and in two electrode vessels.

N/7 NaOH, warming on a steam bath for a couple of hours, and titrating back with N/7 HCl using phenolphthalein.

β -Hydroxybutyric acid analyzed.....	per cent 100
Lactic acid analyzed.....	95.2

Experiment II. Curve C.—The acids used in the preceding experiment had not been treated to remove any anhydride that might have been present; the experiment was therefore repeated.

TABLE VIII.

Effect of Organic Acids on the Titration Curve.

Normality of organic acid on basis of plasma volume only.	A 1 vol. plasma + 1 vol. N/50 acid. Curve A.	B 1 vol. plasma + 2 vol. N/50 acid. Curve B.
β-Hydroxybutyric acid (Fig. 5).		
	<i>pH</i>	<i>pH</i>
0	6.73	5.34
	6.72	5.35
0.0094	6.55	5.16
	6.55	5.16
0.024	5.84	4.85
	5.84	4.85
0.047	5.08	4.58
	5.07	4.57
0.094	4.59	4.29
	4.58	4.28
0.189	4.15	4.00
		4.00
Lactic acid (Fig. 6).		
0.011	6.26	4.94
	6.24	4.94
0.028	5.355	4.565
	5.345	4.575
0.056	4.58	4.18
		4.18
0.11	4.03	3.83
	4.02	3.83
0.22	3.64	3.48
	3.65	3.48

0.225 gm. of lactic acid was placed in a flask with 30 cc. of N/10 sodium hydroxide, placed on the steam bath for 2 hours, 50 cc. of N/10 HCl were added, and the solution was diluted to 100 cc. The solution was then N 0.025 to lactic acid and N 0.02 to hydrochloric acid.

2 gm. of CaZn β -oxybutyric acid salt were treated with three-fourths of its equivalent sulfuric acid and the liberated acid was extracted with ether in a wet extractor. The ether was evaporated off over water and the solution diluted to 100 cc. The amount of acid was titrated with sodium hydroxide as in lactic acid. 0.130

gm. of this acid was then treated with 15 cc. of $N/10$ sodium hydroxide heated on the steam bath for 2 hours, 25 cc. of $N/10$ HCl were added, and the solution was diluted to 50 cc. This solution was then $N\ 0.025$ to β -oxybutyric acid and $N\ 0.02$ to hydrochloric acid.

TABLE IX.

Effect of Organic Acid on the Titration Curve.

Solution A. 1 vol. plasma + 2 vol. $N/50$ HCl.	Solution B.*	Normality of organic acid on basis of plasma volume only.	Lactic acid. Curve C, Fig. 6.	β -Hydroxybutyric acid. Curve C, Fig. 5.
—	0	0	<i>pH</i> 4.55	<i>pH</i> 4.55
4.0	0.5	0.0055	4.41	4.45
4.0	1.0	0.01	4.32	4.39
4.0	2.0	0.0166	4.20	4.31
3.0	3.0	0.025	4.09	4.24
1.0	3.0	0.037	3.96	4.15
0	—	0.05	3.85	4.07

* Solution: β -oxybutyric acid or lactic acid, $N\ 0.025$; hydrochloric acid, $N\ 0.02$.

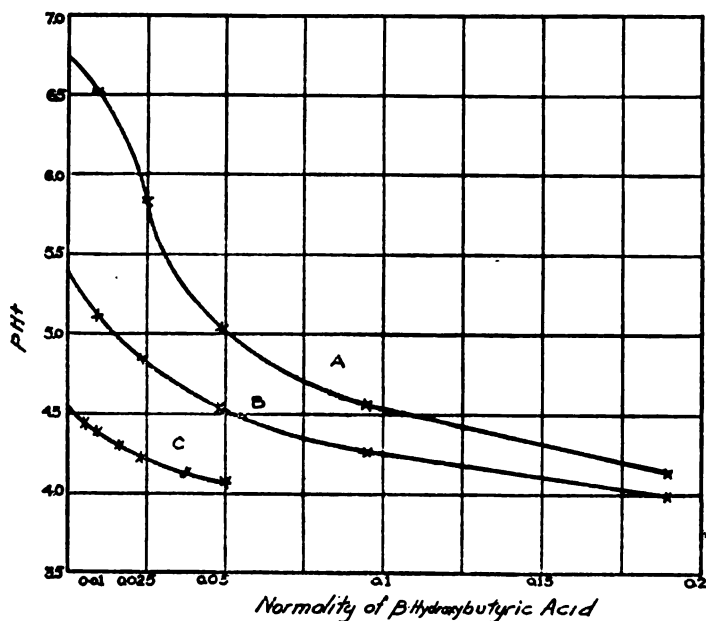


FIG. 5.

The normality figures in A and B do not represent actual amounts of free acid due to the presence of undissociated anhydride, but they do indicate the change in the titration curve caused by the accumulation of such acid. Curve C indicates the nature of the decreased reserve in a dog plasma with very low reserve. The normality figures here represent actual values.

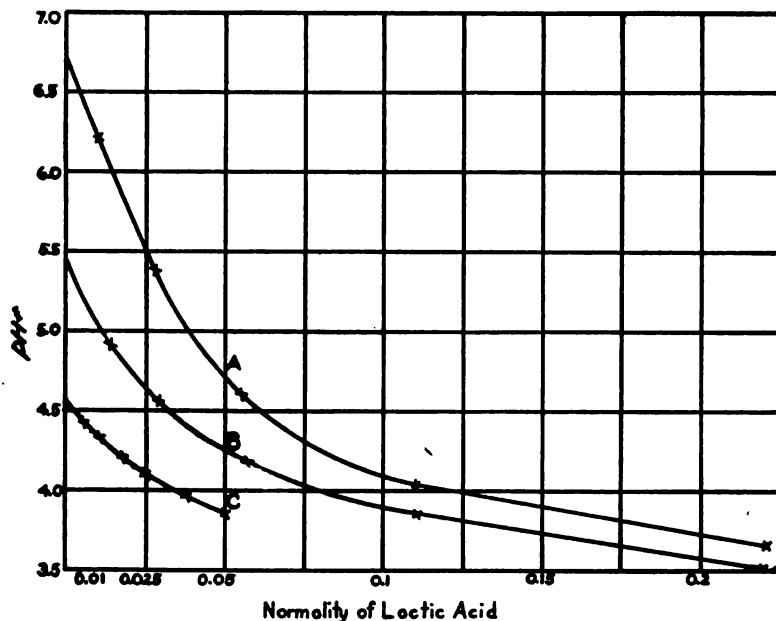


FIG. 6.

Range of Normal Values.

Table X shows the variations in normal plasma in different individuals at different times. The values in a healthy man may change on titration with one volume of $N/50$ acid from a pH of 6.75 to 7.00 during the course of the day. The corresponding figures for titration with two volumes of $N/50$ acid are from a pH of 5.60 to 5.90. Dogs have a lower reserve averaging around a pH of 5.00.

The normal base line for titration with one volume of $N/50$ acid in the accompanying charts is taken at 7.00; with two volumes at 5.6. The normal limits for the "carbon dioxide combining capacity"

is from 55 to 70. The normal alveolar carbon dioxide tension in mm. is about 38. From a number of comparisons the ratio between plasma carbon dioxide volume per cent and alveolar carbon dioxide tension in mm. has been established as 1.45 (see Paper VI).

TABLE X.

Table of Normal Variations.

Procedure A. 1 vol. plasma + 1 vol. N/50 acid.			Procedure B. 1 vol. plasma + 2 vol. N/50 acid.		
Plasma.	Time.*	pH.	Plasma.	Time.	pH.
M.	a.m.	6.80	Dog 1.	a.m.	4.81
	p.m.	6.80		p.m.	5.02
V.	May 15, a.m.	7.04	" 2.	a.m.	5.18
	p.m.	6.89		p.m.	5.24
	" 17, a.m.	6.96	" 3.	a.m.	4.91
	p.m.	6.94		p.m.	5.23
D.	a.m.	7.10	M.	May 18	5.50
	p.m.	7.60		" 22	5.63
P.	a.m.	6.73	F.		5.69
	p.m.	6.84	P.		5.50
S.	a.m.	6.65			
	2 p.m.	6.74			
	4 "	6.71			
	6 "	6.82			
C.	May 19, a.m.	6.58			
	p.m.	6.83			
	"	6.83			
	" 22, p.m.	6.85			

* Indicates before and after meals.

Application of the Method.

The charts of diabetic patients followed for a long period by the electrometric titration of plasma, simultaneously with the determination of CO₂ combining capacity of blood and plasma, alveolar air, and acid excretion, are given in Paper VI of this series. The values of simultaneous determination of the "carbon dioxide combining

capacity" and alveolar carbon dioxide are also shown, since it was one purpose of this series of observations to select the most suitable method for determining the actual amount of "alkaline reserve" in the body.

The samples of blood were obtained from the arm, were drawn through a McRae needle into tubes containing a small amount of potassium oxalate, and centrifuged at once. The determinations were, with a few exceptions, done at once, but samples may be kept for a considerable time in paraffined tubes.

The observations were divided into two periods; the first covering the spring of 1915, the second that of 1916. The electrometric titrations on the first series were done by procedure A—1 volume of plasma + 1 volume of $N/50$ HCl; on the second series by procedure B—1 volume of plasma + 2 volumes of $N/50$ HCl. This eliminated the theoretical objection of possible error due to the presence of carbon dioxide, but proved to have the disadvantage of lessening the sensitivity of the titration, in that for given decrease in carbon dioxide combining power the corresponding change in pH was less than with one volume of acid.

CONCLUSIONS.

The conclusions are evident from the charts. The two methods of determining the "alkaline reserve" are entirely comparable and furnish a reliable index of the reserve actually existing in the body. The titration of the plasma includes the influence of all the "buffers," not only the sodium bicarbonate, but also the proteins and the minute amounts of phosphates. The fact that the electrometric titration of the plasma gives results parallel with the carbon dioxide combining capacity indicates that the latter is proportional to the total "buffer" content of the plasma.

SUMMARY.

1. The gas chain method of determining hydrogen ion concentration has been utilized in the titration of plasma and the conditions for successful operation have been determined.
2. Values for normal and pathological plasmas have been determined.

3. The method has been compared with that of the carbon dioxide combining capacity in a long series of diabetic patients, and the close agreement of the two methods has been established.

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STUDIES OF ACIDOSIS.

IV. THE RELATIONSHIP BETWEEN ALKALINE RESERVE AND ACID EXCRETION.

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The object of the work presented in this paper was to ascertain whether in diabetes a quantitative relationship could be discovered between the alkaline reserve of the blood plasma, as measured by its combining power for CO_2 , and the rate of acid excretion by the kidneys. It has been known since the time of Walter¹ that entrance of acid into the circulation immediately reduces the blood bicarbonate, and is accompanied by an increased rate of acid and ammonia excretion in the urine. A quantitative relationship between decreased blood bicarbonate and acid excretion has not been demonstrated.

To undertake this problem the plasma bicarbonate was estimated in volumes per cent by the method described in Paper I of this series and compared with the excretion of acid in the urine in the following way. The ammonia of the urine was added to the acid titratable with phenolphthalein by Folin's method² as a measure of the excretion of acids in excess of mineral bases, the whole being expressed as 0.1 N acid. The greater part of such excess acid is usually neutralized by ammonia in man, but as shown by Henderson and Palmer³ an acid as weak as β -hydroxybutyric can be excreted 45 per cent free, while more than 80 per cent of the phosphate can be excreted as the acid NaH_2PO_4 . Consequently in order to determine the total excretion of acid in excess of mineral bases the free titratable acid must be added to that neutralized by ammonia.

¹ Walter, F., *Arch. exp. Path. u. Pharm.*, 1877, vii, 148.

² Folin, O., *Am. J. Physiol.*, 1903, ix, 265.

³ Henderson, L. J., and Palmer, W. W., *J. Biol. Chem.*, 1913, xiv, 81.

The Folin method for titration of free acid was chosen because the acid titratable with phenolphthalein approaches zero in human urine when the height of the plasma bicarbonate is at its maximum normal of about 80 volume per cent, under which conditions ammonia excretion also approaches zero.

Comparison of the amounts of ammonia plus acid excreted per 24 hours in a number of diabetics followed over considerable periods in the Rockefeller Hospital indicated a general relationship between daily excretion and the plasma bicarbonate content. It was impossible, however, to form any estimate of the plasma bicarbonate from the rate of excretion alone, and the ordinary $\frac{\text{NH}_3}{\text{total N}}$ ratio was even less satisfactory. As Folin showed, this ratio may be greatly increased beyond the usual value by merely reducing the protein intake and consequently the denominator of the ratio; and quite aside from variations that could be explained by this cause, the ammonia ratio bore only the most casual quantitative relationship to the plasma bicarbonate.

In the meantime, however, one of us (F.) working on diabetic metabolism at the Peter Bent Brigham Hospital had used the Ambard formula to compare the acid excretion with the blood alkali as indicated by the alveolar CO_2 tension. Ambard and Weill⁴ found that in the cases of urea and chloride, excretion rate is proportional to the square of the concentration of the excretory substances in the blood above the excretion threshold, to the square root of the volume of water eliminated, and to the first power of the weight of the subject. (For a complete discussion see McLean.⁵) The above facts are reduced to algebraic form by the equation:

$$(1) \text{ Blood concentration} = \text{constant} \times \sqrt{\frac{D}{W}} \sqrt{C}$$

D is the excretion rate, W the body weight, and C the concentration of excretory product in the urine. The expression $\sqrt{\frac{D}{W}} \sqrt{C}$ is proportional to the blood concentration of the excretory product above the excretion threshold.

⁴ Ambard, L., *Physiologie normale et pathologique des reins*, Paris, 1914.

⁵ McLean, F. C., *J. Exp. Med.*, 1915, xxii, 212.

If acid excretion should follow the same law, a similar expression might be expected to indicate the degree of acid retention in the blood. In order to test this possibility, the rate of excretion of 0.1 N acid + NH_3 (24 hour time unit) was taken as D and the cc. of 0.1 N acid + NH_3 per liter of urine as C . Assuming that acid accumulation in the plasma is proportional to the fall of the plasma CO_2 figure below the maximum figure of 80, we may express the relation between the blood accumulation and acid excretion as follows:

$$(2) \text{ Retained acid} = 80 - \text{plasma } \text{CO}_2 = \text{constant} \times \sqrt{\frac{D}{W}} \sqrt{C}$$

The constant of the equation in repeated observations proved to be 1, so that the relationship between plasma CO_2 capacity and acid excretion may be expressed more simply as:

$$(3) \text{ Plasma } \text{CO}_2 \text{ capacity} = 80 - \sqrt{\frac{D}{W}} \sqrt{C}$$

This formula is purely empirical, and it was not adopted until tests upon some scores of urines had shown that it held more consistently than any other expression which could be found in the literature or devised. As stated before, the rate of excretion alone, as well as the ammonia : total nitrogen ratio failed to show consistent relationship to the blood bicarbonate. No other equation including excretion rate and concentration was so satisfactory as Ambard's. If the square root of the whole were not taken the urine index varied much more than the blood. If the expression $\sqrt{\frac{DC}{W}}$ were used, the influence of concentration (C) was given too much weight, while $\sqrt{\frac{D}{W}} \sqrt[3]{C}$ gave too little influence to C . In short, although reasoning by analogy led to the trial of the Ambard equation, it has been retained solely because it expresses the relationship between blood alkali and acid excretion more accurately than any other expression at present available.

As in Ambard's tests, the value of D and C may be determined upon a 24 hour specimen of urine or upon a shorter period from which the

24 hour rate of excretion can be computed. In the cases reported in this paper short periods were chosen, varying in length from 1 to 4 hours. The advantage of short periods in tests of this description has been pointed out by McLean. By collecting the urine over a given period and withdrawing blood at the middle of it, the blood sample may be assumed to represent the average for the period. If no food or water is taken during the period, and the latter is not too soon after a heavy meal, the rate of excretion during the period will remain practically constant.

Experiments were conducted upon normal individuals to determine the degree of constancy of the relationship expressed in Equation 3 above. Different normal individuals were tested at different times, and a few were given sodium bicarbonate to discover at what level of blood bicarbonate content, if any, the excretion of acid in the urine ceased. The results of these observations are tabulated in Table I.

A certain margin of error must be accepted in calculating the internal concentration of any substance from the excretion, and the margin in this instance appears to be about 10 volumes per cent of plasma CO_2 . In the normal cases the maximum variations encountered were 8.7 volumes per cent too high, and 6.7 volumes per cent too low.

Thirty-six cases of diabetes were tested in the manner described, selected as they reported to the hospital. Nothing was known about their previous treatment or condition except that a few stated that they had been taking sodium bicarbonate before the test was made. The results are tabulated in Table II.

Of the 36 cases, 26 showed values of the index from which the plasma CO_2 could be estimated with an error not greater than 10 volumes per cent. Of the remaining 10, 4 showed normal plasma CO_2 , the CO_2 calculated from the urine being merely a higher normal. Six cases of acidosis remain in which an error greater than 10 volumes per cent was made in estimating the plasma bicarbonate from the acid excretion. In four of them bicarbonate had been administered, and the plasma bicarbonate was found much higher than indicated by the urine index. This is usually the case after bicarbonate administration, the urine failing to show the full effect of the alkali

TABLE I.
Excretion of Acid Compared with the Plasma Bicarbonate in Normal Individuals.

Subject.	Date.	Time.	Weight. kg.	Urine measured.	Volume calculated to 24 hrs.	0.1 N NH ₃ per liter.	0.1 N acid per liter.	C	D	$\sqrt{\frac{M}{D} \sqrt{C}}$	CO ₂ bound by 100 cc. of plasma.		Difference.	NaHCO ₃ gm.
											Found.	Calculated by $80 - \sqrt{\frac{M}{D} \sqrt{C}}$		
McL.	Feb. 19, 1916	60	81	285	6,850	70	90	160	1,096	13.1	62.7	66.9	+4.2	
F.	Oct. 28, 1915	120	70	130	1,560	100	266	366	571	12.6	65.0	67.4	+2.4	
P.	Dec. 1, 1915	72	89	34	680	592	415	1,007	684	15.6	65.8	64.4	-1.4	
S.	Nov. 16, 1915	66	86	49.5	1,080	387	416	803	867	16.9	68.3	63.1	-5.2	
F.	Dec. 1, 1915	72	70	84	1,680	252	110	362	652	13.3	69.5	66.7	-2.8	
McL.	Jan. 27, 1916	60	81	375	9,000	45	24	69	621	2.5	69.8	77.5	+7.7	
G.	Oct. 28, 1915	72	82.8	65	1,300	110	100	210	273	6.9	71.6	73.1	+1.5	3
V. S.	Nov. 13, 1915	30	70	27	1,297	76	40	116	150	4.8	72.5	75.2	+2.7	
McL.	Jan. 27, 1916	60	81	167	4,000	19	64	83	332	1.9	73.9	78.1	+4.2	17
V. S.	Nov. 16, 1915	60	70	70	1,440	135	28	163	235	6.5	75.2	73.5	-1.7	
V. S.	Feb. 21, 1916	60	70	—	—	—	—	—	—	11.7	75.3	68.6	-6.7	5
V. S.	" 21, 1916	60	70	—	—	—	—	—	—	3.0	78.0	77.0	-1.0	
McL.	" 19, 1916	60	81	68.5	1,645	6	0	6	10	0.6	79.3	79.4	+0.1	20

TABLE II.
Excretion of Acid Compared with the Plasma Bicarbonate in 36 Cases of Diabetes.

No.	Subject.	Date.	Time.	Weight. kg.	Urine measured. cc.	Volume calculated to 24 hrs. cc.	0.1 N NH ₃ per liter. cc.	0.1 N acid per liter. cc.	C	D	$\sqrt{\frac{M}{D}} \sqrt{C}$	CO ₂ bound by 100 cc. of plasma.			Difference.
												Round.	Calculated by $80 - \sqrt{\frac{M}{D}}$	cc.	
1*	Z.	Mar. 14, 1917	4 hrs.	23.0	590	3,540	400	244	644	2,280	50.2	14.0	29.8	cc.	+15.4
2	C.	June 23, 1916	72 min.	37.3	173	3,460	825	328	1,153	4,000	60.3	22.5	19.7	cc.	-2.8
3	H.	" 10, 1916	72 "	42.0	140	2,800	1,160	280	1,440	4,040	60.4	29.2	19.6	cc.	-9.6
4†	S.	Feb. 15, 1917	72 "	43.0	175	3,500	1,070	430	1,500	5,250	68.8	31.0	11.2	cc.	-19.8
5	E.	Nov. 18, 1915	72 "	52.2	175	3,500	900	320	1,220	4,270	53.4	33.6	26.6	cc.	-7.0
6	F.	Dec. 21, 1915	2 hrs.	45.0	500	6,000	252	335	587	3,522	43.5	33.6	36.5	cc.	+2.9
7	H.	Nov. 22, 1916	72 min.	50.0	306	6,120	612	247	859	5,260	55.5	34.0	24.5	cc.	-9.5
8†	W.	" 21, 1916	72 "	50.0	175	3,500	1,056	431	1,487	5,200	63.3	35.0	16.7	cc.	-18.3
9	L.	Feb. 1, 1917	72 "	43.8	535	10,700	160	122	282	3,018	34.0	36.5	46.0	cc.	+9.5
10†	O.R.	" 14, 1917	72 "	32.8	145	2,900	1,116	438	1,554	4,500	73.5	41.0	6.5	cc.	-34.5
11	A.	Sept. 6, 1916	72 "	42.2	285	5,700	374	266	640	3,642	46.7	42.1	33.3	cc.	-8.8
12	L.	June 8, 1916	72 "	50.0	127	2,540	671	352	1,023	2,600	40.8	42.8	39.2	cc.	-3.6
13	O.B.	Feb. 23, 1917	72 "	37.2	56	1,120	1,088	496	1,584	1,775	43.6	47.0	36.4	cc.	-10.6
14	M.	Aug. 24, 1916	72 "	37.0	104	2,080	450	402	852	1,770	37.4	47.2	42.6	cc.	-4.6
15	C.	Nov. 11, 1915	2 hrs.	47.0	140	1,680	506	152	658	1,080	24.3	47.8	55.7	cc.	+7.9
16†	D.	Jan. 2, 1916	72 min.	28.8	118	2,360	1,100	425	1,525	3,600	69.9	47.8	10.1	cc.	-37.7
17	C.	Oct. 30, 1915	72 "	60.0	145	2,900	268	260	528	1,531	24.2	50.6	55.8	cc.	+5.2

18	C.	Aug. 29, 1916	72 min.	52.0	229.5	4,590	—	—	335	1,538	23.3	50.7	56.7	+ 6.0
19	H.	Oct. 11, 1916	72 "	56.1	375	7,500	182	164	346	2,590	29.3	50.8	50.7	- 0.1
20	U.	" 19, 1915	72 "	46.5	87	1,740	668	296	964	1,677	33.5	51.1	46.5	- 4.6
21	P.	Nov. 11, 1915	72 "	46.0	305	6,100	168	64	232	1,415	23.2	52.5	56.8	+ 4.3
22	D.	" 4, 1915	72 "	48.7	110	2,200	172	575	747	1,644	20.4	53.3	59.6	+ 6.3
23	K.*	" 11, 1915	72 "	28.5	40	800	468	130	598	478	20.2	53.5	59.8	+ 6.3
24	R.	June 16, 1916	72 "	49.2	270	5,400	88	94	182	985	16.4	54.7	63.6	+ 8.9
25	S.	Dec. 8, 1915	72 "	42.6	83	1,660	144	50	194	320	10.2	55.1	69.8	+14.7
26	D.	Oct. 21, 1915	72 "	50.0	100	2,000	264	316	580	1,160	23.6	55.4	56.4	+ 1.0
27	R.	Nov. 18, 1915	3 hrs.	87.2	360	2,880	328	305	633	1,822	23.0	56.2	57.0	+ 0.8
28	S.	Dec. 1, 1915	2½ "	50.4	520	5,000	172	105	277	1,390	21.4	56.4	58.6	+ 2.2
29	C.	Sept. 28, 1916	72 min.	40.0	600	12,000	171	28	199	2,400	29.1	57.9	50.9	- 7.0
30	S.	" 28, 1916	72 "	51.0	175	3,500	222	146	368	1,290	22.0	57.9	58.0	+ 0.1
31	H.	Nov. 11, 1915	2 hrs.	47.8	340	4,080	44	24	68	278	6.9	58.2	73.1	+14.9
32	P.	" 15, 1915	1½ "	54.0	100	1,694	120	140	260	441	11.5	59.5	68.5	+ 9.0
33	S.	June 8, 1916	72 min.	25.8	135	2,700	313	50	363	980	26.9	59.8	53.1	- 6.7
34	W.	Aug. 24, 1916	72 "	67.8	42	840	150	129	279	234	6.8	61.0	73.2	+12.2
35	M.	Nov. 11, 1915	2 hrs.	50.2	150	1,800	108	28	136	245	7.5	61.4	72.5	+11.1
36	C.	June 13, 1916	72 min.	39.8	64	1,280	418	64	482	616	18.4	69.2	61.6	- 7.6

*Coma.

†NaHCO₃.

TABLE III.
Excretion of Acid Compared with the Plasma Bicarbonate in 29 Observations on the Same Case.

No.	Date.	Time.	Weight.	Urine measured.	Volume calculated to 24 hrs.	0.1 N NH ₃ per liter.	0.1 N acid per liter.	C	D	$\sqrt{\frac{D}{W}} \sqrt{C}$	CO ₂ bound by 100 cc. of plasma.		Difference.	NaHCO ₃
											Found.	Calculated by $80 - \sqrt{\frac{D}{W}} \sqrt{C}$		
	1915	min.	kg.	cc.	cc.	cc.	cc.							gm.
1	Oct. 16	72	48.0	100	2,000	1,242	540	1,782	3,564	56.0	29.0	24.0	- 5.0	
2	" 17	72	48.4	100	2,000	876	332	1,208	2,416	41.6	41.6	39.4	- 2.2	
3	" 19	72	48.7	110	2,200	828	216	1,044	2,297	39.0	43.6	41.0	- 2.6	
4	Nov. 8	72	46.8	200	4,000	204	85	289	1,156	20.5	45.9	59.5	+13.6	
5	" 19	2½ hrs.	48.0	59	531	1,352	335	1,687	896	27.7	47.9	52.3	+ 4.4	
6	" 9	72	46.8	130	2,600	448	190	638	1,659	29.9	48.4	50.1	+ 1.7	
7	Oct. 16	1 hr.	48.0	178	4,272	154	312	466	1,991	29.9	48.6	50.1	+ 1.5	
8	Nov. 22	2 hrs.	48.2	70	774	780	205	985	685	23.7	48.6	56.3	+ 7.7	50
9	" 5	72	47.8	55	1,100	912	595	1,507	1,658	36.7	48.9	43.3	- 5.6	
10	" 12	72	47.0	35	700	1,168	450	1,618	1,133	31.1	49.3	48.9	- 0.4	
11	" 4	72	47.8	40	800	896	480	1,376	1,101	29.2	51.1	50.8	- 0.3	
12	" 13	72	46.7	105	2,100	568	220	788	1,655	31.5	51.5	48.5	- 3.0	
13	" 10	72	47.5	175	3,500	316	130	446	1,561	26.3	52.5	53.7	+ 1.2	
14	Oct. 23	72	49.5	55	1,100	1,057	372	1,429	1,572	34.2	52.7	46.0	- 6.7	
15	Nov. 24	2½ hrs.	48.6	125	1,333	360	75	435	578	15.7	54.5	64.3	+ 9.8	
16	Oct. 27	72	50.1	85	1,700	400	215	615	1,045	22.7	54.9	57.3	+ 2.4	
17	Nov. 29	72	49.4	18	360	880	300	1,180	425	17.2	55.5	62.8	+ 7.3	
18	" 15	72	46.8	20	400	1,069	240	1,309	428	18.2	55.6	61.8	+ 6.2	
19	" 3	72	48.0	75	1,500	536	280	816	1,224	27.0	55.7	53.0	- 2.7	

20	Oct. 29	72	49.3	470	9,400	72	40	112	1,052	8.4	56.9	71.6	+14.7	30
21	" 20	72	49.4	134	2,680	596	200	796	2,134	34.9	57.2	45.1	-12.1	
22	" 22	72	49.6	291	5,820	230	92	332	1,932	26.6	57.8	53.4	- 4.4	
23	" 25	72	50.5	245	4,900	240	88	328	1,607	24.0	57.9	56.0	- 1.9	
24	" 28	72	50.0	360	7,200	124	80	204	1,469	20.5	58.4	57.5	- 0.9	
25	" 26	72	50.3	220	4,400	164	52	216	948	16.6	59.0	63.4	+ 4.4	30
26	" 18	72	49.5	340	6,800	332	168	500	3,400	39.2	60.0	40.8	-19.2	
27	Nov. 2	72	48.7	30	600	784	380	1,164	698	22.1	60.6	57.9	- 2.7	
28	" 1	72	48.8	130	2,600	148	44	192	499	11.9	60.8	68.1	+ 7.3	30
29	Oct. 21	72	50.0	250	5,000	288	128	416	2,080	29.1	64.0	50.9	-13.1	

on the blood. Of the remaining two cases, one was in coma, and the excretory mechanism was presumably affected. The other case was one of mild acidosis (47.0 per cent plasma CO_2) which gave an index indicating 36.4 per cent, a decidedly more severe condition. Summarized, the results indicate that in diabetic patients short of coma and not receiving bicarbonate the state of the plasma bicarbonate CO_2 can usually be estimated within 10 volumes per cent from the index of acid excretion.

In order to study variations in the same individual, twenty-nine observations were made in the course of a few months upon a patient who entered the hospital with severe acidosis. The result of these repeated tests is shown in Table III.

Of these twenty-nine observations, in five the calculated plasma bicarbonate differed by more than 10 volumes per cent from that found. Three of these discrepancies seemed to depend on the fact that sodium bicarbonate had been given. These observations, like those in Table II, show that sodium bicarbonate disturbs the relationship between blood and urine, the latter failing to show the extent to which the alkali raises the plasma CO_2 capacity.

Use of Curves to Calculate the Index.

In order to simplify the calculation of the index we have prepared a set of curves which enable one by mere inspection to estimate the index with sufficient accuracy, the only data required being (1) the cc. of urine excreted per 24 hours per kilo body weight, and (2) the cc. of 0.1 N NH_3 + titratable acid per liter of urine.

Since D , the rate of excretion per 24 hours, is equal to the product VC (V = volume of urine per 24 hours in liters) the expression $\sqrt{\frac{D}{W}} \sqrt{C}$ may be written

$\sqrt{\frac{VC}{W}} \sqrt{C} = \left(\frac{V}{W}\right)^{\frac{1}{2}} C^{\frac{1}{2}}$. Consequently one can plot on coordinate paper the values of the index corresponding to different values of $\frac{V}{W}$ and C .

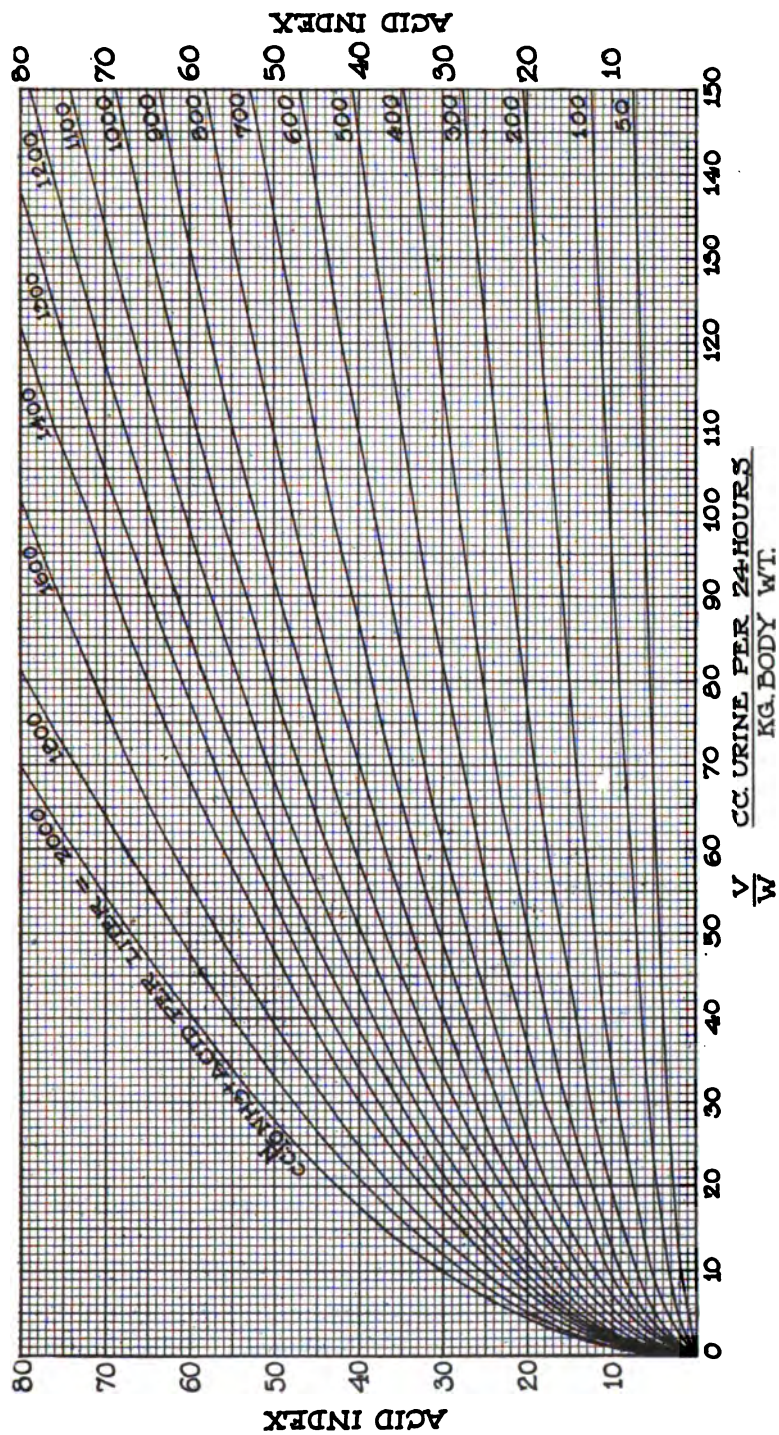


FIG. 1.

The accompanying figure indicates the manner in which it appeared simplest to do this.

To use an example: If in a given case the body weight is 50 kg., the volume of urine per 24 hours 2,000 cc., and the NH_3 + titratable acid 1,200 cc. per liter, we have the two values $\frac{D}{W} = \frac{2,000}{50} = 40$ cc. of urine per 24 hours per kg., and $C = 1,200$. We run down the curve corresponding to $C = 1,200$ until it is crossed by the vertical line corresponding to $\frac{V}{W} = 40$, and read off 41 as the value of the index. If the value of C were 1,250, we should estimate one-half of the distance between the 1,200 and 1,300 curves of C , and read 42 as the value of the index.

SUMMARY.

In normal men and diabetics the excretion of acid in excess of fixed bases as measured by determining ammonia plus titratable acid bears a quantitative relationship to the alkaline reserve of the body as measured by the CO_2 binding power of the blood plasma.

Such relationship is demonstrated by the use of an empirical expression of the form devised by Ambard to denote the relationship between blood concentration and excretion in the cases of salt and

urea. The formula used is $\sqrt{\frac{D}{W}} \sqrt{C}$, D representing the rate of excretion of 0.1 N ammonia + titratable acid per 24 hour time unit, C the 0.1 N NH_3 + acid per liter of urine, and W the body weight.

The value $80 - \sqrt{\frac{D}{W}} \sqrt{C}$ determined in the urine indicates with an error which is usually less than 10 volumes per cent, the level of the plasma CO_2 capacity. Diabetics receiving bicarbonate administrations are exceptions, the blood bicarbonate in such cases being, as a rule, much higher than indicated by the urine. One case in coma showed a much lower blood bicarbonate than was indicated by the urine.

The index can be determined from analysis of the urine passed in 24 hours or from the amount excreted in 1 or 2 hours, multiplied to bring the data to a 24 hour basis. The calculation is simplified by the

use of curves, which make it possible to estimate the index from inspection, the two necessary figures being the cc. of urine passed per kilo body weight per 24 hours, and the amount of ammonia plus titratable acid per liter of the urine.

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STUDIES OF ACIDOSIS.

V. ALVEOLAR CARBON DIOXIDE AND PLASMA BICARBONATE IN NORMAL MEN DURING DIGESTIVE REST AND ACTIVITY.

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Higgins¹ and Erdt² have both found that digestive activity causes a rise in alveolar carbon dioxide tension. Their explanations of this phenomenon are different. Erdt believed that the change was due to an increase in the reserve alkali of the blood, caused by secretion of hydrochloric acid in the gastric juice. Higgins, however, had in mind the readiness with which slight changes in conditions can affect the alveolar carbon dioxide, without any conceivable effect on blood alkali. For example, he found that changing the posture from standing to lying increased the alveolar carbon dioxide tension by 6 mm., apparently by rendering the respiratory center less irritable. He believed that the effect of a meal was due to a similar influence on respiration, rather than on the alkaline reserve.

It seemed that clarifying evidence might be gained by determining both alveolar carbon dioxide tension and the plasma bicarbonate (under definite carbon dioxide tension as described in Paper I), before and after eating. If the respiratory center remains normal the plasma bicarbonate must rise with the alveolar carbon dioxide, and the ratio $\frac{\text{plasma CO}_2}{\text{alveolar CO}_2}$ remain constant. If, however, the irritability of the center falls, the ratio should fall after meals, the alveolar carbon dioxide increasing, the plasma carbon dioxide either remaining constant, or increasing less in proportion than the alveolar.

¹ Higgins, H. L., *Am. J. Physiol.*, 1914, xxiv, 114.

² Erdt, H., *Deutsch. Arch. klin. Med.*, 1915, xlvii, 497.

Partly to throw light on this question, and partly to obtain statistics on the normal range of plasma bicarbonate values, we have performed these determinations on a number of obliging colleagues. In some cases breakfast, a mixed diet meal taken at about 8 o'clock, was the object of the experiment; in other cases lunch, taken from 4 to 5 hours later. The determinations were made within a half hour before the meal in each case, and between 30 and 60 minutes after it. The blood samples were drawn immediately after the alveolar air samples were taken. The alveolar carbon dioxide samples were taken standing, the Fridericia³ apparatus being used.

The results are tabulated below.

TABLE I.
Observations before and after Breakfast.

Subject.	Time (before and after meal).	CO ₂ bound as bicarbonate by 100 cc. of plasma.	Alveolar CO ₂ .	$\frac{\text{Plasma CO}_2}{\text{Alveolar CO}_2}$
		<i>cc.</i>	<i>mm.</i>	
P.	Before.	68.7	38.7	1.78
	After.	73.5	40.9	1.80
S.	Before.	66.9	41.7	1.60
	After.	63.5	43.9	1.45
M.	Before.	65.4	39.5	1.66
	After.	65.4	43.1	1.52
F.	Before.	68.2	42.8	1.59
	After.	69.1	43.1	1.60

³ Fridericia, L. S., *Berl. klin. Woch.*, 1914, li, 1268.

TABLE II.
Observations before and after Midday Meal.

Subject.	Time (before and after meal).	CO ₂ bound as bicarbonate by 100 cc. of plasma.	Alveolar CO ₂ .	Plasma CO ₂ / Alveolar CO ₂
		cc.	mm.	
McL. I	Before.	53.4	40.6	1.31
	After.	56.1	49.3	1.27
" II	Before.	56.5	41.6	1.36
	After.	61.0	47.3	1.29
" III	Before.	59.1	40.3	1.47
	After.	61.1	41.7	1.47
P.	Before.	65.1	37.8	1.72
	After.	67.2	40.2	1.68
V. I	Before.	75.0	46.3	1.62
	After.	70.8	47.2	1.50
" II	Before.	72.8	44.0	1.66
	After.	69.8	43.9	1.59
D.	Before.	63.9	45.7	1.40
	After.	68.1	49.8	1.37
S.	1 hr. Before.	68.4	44.0	1.55
	0.5 " After.	63.0	46.1	1.37
	1.5 " "	66.2	48.6	1.36
	2.5 " "	68.6	47.3	1.45
C.	1 hr. Before.	68.2	46.2	1.48
	0.5 " After.	70.7	48.0	1.47
	2.5 " "	70.7	45.1	1.57

DISCUSSION OF RESULTS.

Normal Range of Plasma Carbon Dioxide Capacity.—This series, together with the thirty analyses performed with the same method by Gettler and Baker,⁴ may be taken as establishing the range of plasma carbon dioxide capacity in normal adults. All of our results

⁴ Gettler, A. O., and Baker, W., *J. Biol. Chem.*, 1916, xxv, 211.

fall between 53 and 75 cc. of carbon dioxide bound as bicarbonate by 100 cc. of plasma, and all of Gettler and Baker's are between 56 and 78. Consequently the extreme range appears to lie between 53 and 78. Only one plasma out of the fifty-nine failed to bind more than 55 per cent of its volume of CO_2 (McL. I binding 53.4), so that 55 may be taken as the usual minimum.

Normal Relationship between Plasma Bicarbonate and Alveolar Carbon Dioxide.—The ratio $\frac{\text{plasma CO}_2}{\text{mm. alveolar CO}_2}$ varies from 1.27 to

1.80. It shows some tendency toward characteristic levels in given individuals, McL. always showing a low ratio, P. a high one. The average is approximately 1.5. In order to obtain comparison of the alveolar and plasma data, therefore, the alveolar figure may be multiplied by 1.5. Or the plasma figure may be multiplied by $\frac{1}{1.5} =$

0.66 to make it comparable with the alveolar. In making such comparisons, the above variations in the normal ratio must not be forgotten.

Effects of Digestion.—The observation of Higgins and of Erdt is confirmed, that the alveolar carbon dioxide tension rises after a meal. In the one individual in whom a fall was noted (V.), the alveolar carbon dioxide was extremely high in the morning before lunch, probably because of an alkaline vegetarian breakfast.

The plasma bicarbonate in some cases increases slightly, in others does not.

The ratio $\frac{\text{plasma CO}_2}{\text{alveolar CO}_2}$ in four experiments out of thirteen is changed by less than 0.02, which is within the limit of error. In the other nine experiments the ratio is definitely increased. The results therefore favor Higgins' rather than Erdt's explanation as to the chief factor causing increase in alveolar carbon dioxide during digestion, but the nature of the results is such that data on a statistical scale are necessary for a definite decision.

STUDIES OF ACIDOSIS.

VI. THE BLOOD, URINE, AND ALVEOLAR AIR IN DIABETIC ACIDOSIS.

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The work in the present paper constitutes a study of the quantitative measures of acidosis in the blood, urine, and alveolar air of diabetic patients. It has had for its object the accumulation of data sufficient to show: (1) The relationship of plasma bicarbonate deficiency in man to the clinical severity of acidosis; and (2) the relationship of the blood bicarbonate determined directly in the plasma to that estimated indirectly from the excretions of the lungs and kidneys.

The alveolar carbon dioxide tension, under ideal normal conditions, indicates the level of the arterial blood bicarbonate. It has been shown by several authors,¹ however, that the alveolar carbon dioxide tension is readily altered by numerous factors, psychic, pathological, and physiological, besides the blood bicarbonate, and consequently is a reliable measure of the latter only when it is certain that both the mechanical and nervous factors controlling respiration are normal. The alveolar carbon dioxide tension has found its chief clinical application probably in the detection of diabetic acidosis; but so far as we can ascertain no attempt has yet been made to determine whether the quantitative relationship between blood bicarbonate and alveolar carbon dioxide in diabetes is normal or otherwise.

In Paper V we have determined the normal ratio between plasma bicarbonate and alveolar carbon dioxide. In the present paper we have determined the same ratio in twenty-one diabetic patients and

¹ For discussion of the relationship between alveolar carbon dioxide tension and the arterial blood bicarbonate, see Paper I.

one nephritic patient, examined frequently over periods usually of several weeks. We found that in a third of these patients the alveolar carbon dioxide either indicated a bicarbonate deficit when none existed, or indicated a much greater deficit than actually existed. The reverse, *i.e.*, alveolar air too high, was encountered only in the nephritic. The most ready explanation is that the diabetic patients, most of whom were on a low diet, had abnormally irritable respiratory centers.

In Paper IV it has been shown that in most diabetics a quantitative relationship can be demonstrated between the blood plasma bicarbonate and the excretion of ammonia plus titratable acid in the urine.

The excretion is expressed by the index $\sqrt{\frac{D}{W}}\sqrt{C}$ (where D represents the rate of excretion of N/10 ammonia plus acid per 24 hours time unit, W the body weight, and C the N/10 ammonia plus acid per liter). The plasma bicarbonate carbon dioxide falls in proportion as the index rises, the relationship being expressed by the equation:

$$\text{Volume per cent plasma CO}_2 = 80 - \sqrt{\frac{D}{W}}\sqrt{C}$$

Excepting cases treated with bicarbonate, the plasma carbon dioxide in acidosis could, as a rule, be estimated from the urine to within ± 10 volume per cent. It was desirable to obtain further data concerning the regularity of this relationship between blood and urine in diabetes by systematic observations over considerable periods of time on patients in varying stages of acidosis. On sixteen of the twenty-two patients studied we have consequently followed the acid index, together with the other determinations, throughout the periods of observation. Reference to the charts shows that on the whole the urine index of acid excretion, considering its purely empirical nature, agrees surprisingly well with the plasma bicarbonate.

As additional controls on the blood itself, the acid-neutralizing power of the plasma was estimated by the electrometric titration (Paper III) and the carbon dioxide capacity of the whole blood as well as of the plasma was determined. The electrometric titration was performed as a measure of the *total* buffer content of the plasma. The object in determining the carbon dioxide capacity of the whole

blood was to ascertain whether the technique employed in plasma analyses occasioned any errors, caused by loss of carbon dioxide from the blood and consequent effect on the acid-base transfer between plasma and corpuscles (Paper I).

Methods.

Blood samples were drawn from the arm vein into a McRae tube as described in Paper I. A portion of each sample was used at once for determination of the carbon dioxide binding power of the whole blood, and while this determination was being performed the remainder of the blood was centrifuged, the plasma so obtained being used for determining the carbon dioxide binding power and for the electrometric titration.

The alveolar carbon dioxide tension was determined by Fridericia's² modification of the Haldane method. Determinations were, as a rule, repeated until duplicates were obtained agreeing within 0.1 per cent of carbon dioxide. The method was repeatedly checked by determinations on normal individuals. The patients were well trained in their part of the technique and cooperated satisfactorily. We were surprised at the low results given by some patients with normal or but slightly subnormal plasma carbon dioxide capacity, and tested our technique as completely as possible in order to find a source of error, but were able to discover none.

The electrometric titration of the plasma was determined as described in Paper III.

The carbon dioxide bound as bicarbonate by the plasma was determined as described in Paper I.

The carbon dioxide bound as bicarbonate by the whole blood at normal alveolar temperature and tension was determined as follows: Several cc. of freshly drawn blood were placed in a 300 cc. separatory funnel and warmed to 37°C. (thermometer in funnel) in an incubator. The funnel was then filled with an artificial air-carbon-dioxide mixture containing 5.5 per cent of carbon dioxide, and the blood was saturated with this gas by rotating the funnel. The time required to saturate is longer than the 2 minutes which suffice for plasma, and

² Fridericia, L. S., *Berl. klin. Woch.*, 1914, li, 1268.

in order to insure its completeness the second duplicate was usually done after repeating the saturation. The carbon dioxide determinations were made as described in Paper II. A correction of 3 volume per cent was subtracted for carbon dioxide physically dissolved by the blood under the conditions of saturation.

The index of acid excretion in the urine was determined as described in Paper IV. Aliquot parts of the urine passed during the whole 24 hour period were used for the determinations of ammonia and titratable acid.

Urea was determined in the urine, as a measure of the nitrogen metabolism and concentration of normal urinary constituents, by Marshall's urease method as modified by Van Slyke and Cullen.³

Explanation of Charts.

In the cases where all five determinations were performed, the five sets of results obtained are graphically represented by two sets of curves, the blood analyses in one set, on the upper half of the charts, and the urine and alveolar air in another on the lower half. In order to facilitate comparison of the latter with plasma carbon dioxide, its curve is reproduced in the lower as well as in the upper set.

In both sets of curves a straight line is drawn across at the level indicating the minimum normal carbon dioxide capacity, 55, of adult human plasma. When the plasma curve drops below this line, a condition of acidosis is indicated. The curves of the *upper* set should normally run parallel, but not coincide. The capacity of whole blood to combine with 5.5 per cent carbon dioxide at 37° is about 15 volume per cent less than that of plasma at 20°. Consequently the whole blood curve, where there is no acidosis, may be expected to run parallel to the plasma curve, but below it by a difference indicating about 15 volume per cent carbon dioxide. As acidosis develops and both curves sink toward zero, this difference, of course, diminishes. With these relationships in mind, one may use the whole blood capacity to check the plasma capacity for irregular drops such as could be caused by large losses of carbon dioxide from the blood

³ Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1914, **xix**, 211; 1916, **xxiv**, 117.

sample, and consequent transfer of HCl from corpuscle to plasma, before the blood is centrifuged (Paper I). Such a drop undoubtedly occurred in one plasma determination (Chart 5, Case 2686, March 3), but apparently in only that one out of the scores that were thus controlled.

The electrometric titration curves are so arranged that the normal and extreme acidosis values approximately coincide with those of the plasma CO_2 curves, but the natures of the determinations are so different that only an approximate parallelism can be expected. This is shown quite consistently.

The curves of the *lower* sets are so arranged that if alveolar carbon dioxide and urine excretion maintain normal relationships with the plasma bicarbonate, all three curves should coincide. The alveolar carbon dioxide tension expressed in mm. is multiplied by 1.45 in order to make it comparable to the plasma carbon dioxide capacity expressed in volume per cent. The ratio in normal men varies from 1.3 to 1.8 (Paper V), so that a fairly wide margin for normal deviations must be allowed. But when the alveolar $\text{CO}_2 \times 1.45$ runs over 10 units below the plasma curve the deviation must be taken as abnormal.

The urine index curves also are so plotted that absolute agreement with the plasma results would be indicated by the exact coincidence of the two curves. The degree of deviation in any given case is a measure of the error that would be made in estimating the alkaline reserve of the blood from the acid excretion.

In tabulating the diets, the caloric values when only green vegetables were fed were omitted, because the digestible protein and fat are so uncertain as to make the caloric value indefinite (approximately 6 to 7 calories for each gm. of carbohydrate in green vegetable ingested). When whiskey was given during these periods the caloric value of the alcohol is tabulated.

To the note accompanying each chart is appended the group to which the case belongs according to Stillman, who has classified diabetics as follows⁴:

Group I is composed of cases which maintain a normal bicarbonate reserve of the blood throughout the course of their treatment.

⁴ Stillman, E., *Am. J. Med. Sc.*, 1916, cli, 505.

SUMMARY OF CHARTS AND TABLES.

No.	Case No.	Condition.	Observation period.	Acid excretion index of urine compared with plasma CO ₂ .	Alveolar CO ₂ compared with plasma CO ₂ .
			days		
1	2481	Diabetic with pronounced acidosis during part of period.	31	Not determined.	+*
2	2646	"	22	+*	Alveolar low.
3	2620	"	20	+	" "
4	2680	"	19	+	" "
5	2686	"	22	+	+
6	2414 (1916)	"	25	+	Alveolar low.
7	2414 (1915)	"	49	Not determined.	+
8	2293	"**	4	" "	+
9	2358	"	35	" "	+
10	2684	"	21	+	Alveolar low.
	Feb., 1916				
11	2684	"	23	+	Not determined.
	Jan., 1916				
12	2128 (1916)	"	11	+	" "
13	2382	Diabetic with mild but definite acidosis.	47	Not determined.	+
14	2332	"	36	" "	+
15	2343	"	36	" "	+
16	2128 (1915)	"	33	" "	+
17	2480	Diabetic without acidosis.	19	+	+
18	2234	"	14	+	Alveolar low.
19	2374	"	39	Not determined.	+
20	2389	"	33	" "	+
21	2394	"	34	" "	+
22	2366	Nephritic with acidosis.	61	Not determined.	Alveolar 15 mm. too high.

* The + sign indicates approximate agreement of urine or alveolar air with plasma carbon dioxide.

** Fatal.

In Group II are placed the cases which recover, while fasting, from acid intoxication, sometimes so severe as to verge on coma.

Group III consists of those cases which show a low grade acidosis as indicated by a subnormal bicarbonate reserve and heightened ammonia excretion.

Group IV includes those cases which develop acidosis while fasting, though previously no evidence of acid intoxication existed.

Reference to the charts and tables, and summarizing the general results expressed by them, will be facilitated by use of the table on page 440.

CONCLUSIONS.

The consistent agreement of the carbon dioxide capacity of the plasma and whole blood respectively indicate that, with the technique used for handling blood samples, no significant error is caused by the acid-base transfer between corpuscle and plasma in the period between the drawing and centrifuging of the blood.

The electrometric titration of the plasma roughly parallels the carbon dioxide combining capacity, the agreement showing that the total buffer content of the plasma, determined by the electrometric titration, is approximately proportional to the bicarbonate content.

The alveolar carbon dioxide tension in diabetic patients under treatment is often much too low to indicate the true level of the blood bicarbonate. The alveolar carbon dioxide tension has been observed as low as 26 mm. with a normal plasma bicarbonate. Error in the opposite direction, an alveolar carbon dioxide which is too high, and therefore fails to show an existing acidosis, we have never encountered in diabetes (it is encountered in the one case of nephritis shown in the last chart). When severe diabetic acidosis causes both alveolar carbon dioxide and plasma bicarbonate to fall to a low level, the discrepancy noted above diminishes. When the plasma carbon dioxide has been below 40 volume per cent the agreement between the alveolar and the plasma figures has usually been close.

Excepting those days when bicarbonate was administered, the consistent agreement of the figure for plasma CO_2 , calculated as $80 -$

$\sqrt{\frac{D}{W}} \sqrt{C}$ from Fitz' index of acid excretion in the urine, with the CO_2

determined directly in the plasma is striking. The agreement of urine and blood is on the average more accurate than that of the alveolar air and blood, and is observed not only in adults but also in children of as little as 25 kilos weight.

In very severe acidosis, however, the urine index is less accurate than the alveolar air in indicating the alkaline reserve. With a plasma carbon dioxide of 25 per cent, which corresponds to a urine index of 55, the index may be 65, indicating 15 per cent plasma carbon dioxide, which in our experience is fatal, or it may be 45, indicating 35 per cent plasma carbon dioxide, which though a pronounced acidosis is so well above the danger limit that signs of coma are usually absent.

Of the two indirect measures of alkaline reserve, therefore, the alveolar carbon dioxide appears the more accurate in measuring the more severe stages of diabetic acidosis, such as are encountered in threatened coma, while the index of acid excretion is the more accurate in measuring the more common intermediate stages.

The data published in this and the preceding papers appear sufficiently complete to warrant the generalizations expressed in the following table.

Range of Results Obtained with Normal and Pathological Plasma.

Condition of subject.	Plasma CO ₂ capacity.		Corresponding values of acid index of urine $\sqrt{\frac{D}{W}}\sqrt{C}$ Deviations of ± 10 must be allowed for.
	Reading of apparatus. Gas from 1 cc. plasma.	CO ₂ reduced to 0°, 760 mm., bound as bicarbonate by 100 cc. plasma.	
	cc.	cc.	
Normal resting adult,* extreme limits.....	0.90-0.65	77-53	3-27
Mild acidosis. No visible symptoms.....	0.65-0.52	53-40	27-40
Moderate acidosis. Symptoms may be apparent.....	0.52-0.41	40-30	40-50
Severe acidosis. Symptoms of acid intoxication.....	Below 0.41	Below 30	Over 50
Lowest CO ₂ observed with recovery.....	0.26	16	64

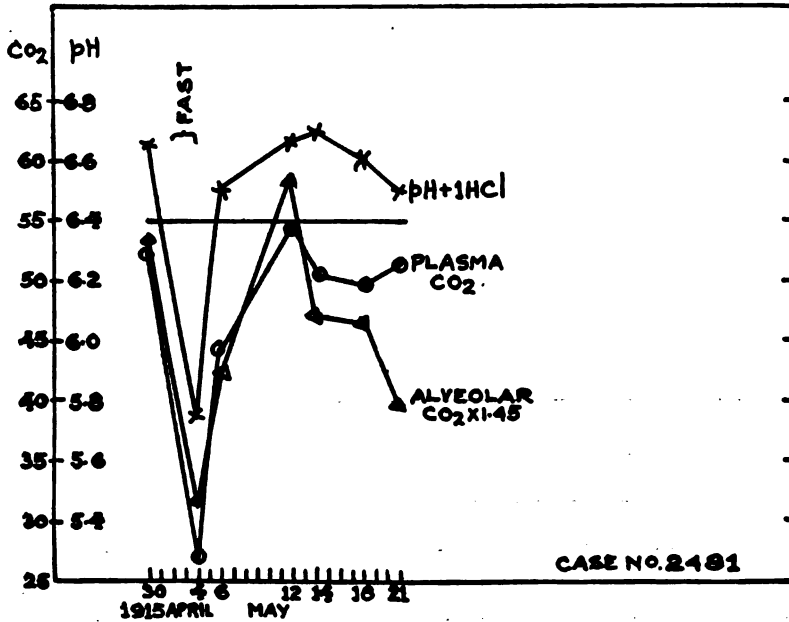
* Schloss (*Am. J. Dis. Child.*, 1917, xiii, 218) finds the carbon dioxide bound by the plasma of normal infants to be 46 to 63 cc. per 100 cc. of plasma, about 10 cc. lower than in adults.

CASES.

1. Case 2481.

Date.	Total Calories.	Protein. gm.	Fat. gm.	Carbohydrate. gm.	Alveolar CO ₂ .		CO ₂ bound by 100 cc. plas- ma at 20°.	Ratio plasma CO ₂ mm. alv. CO ₂	Plasma + 1 vol. N/50 HCl.
					mm.	mm. X 1.45	cc.		
1915 Apr. 30	80	7.5	5.0		37.0	53.6	52.2	1.41	6.66
May 1	160	15.0	10.0						
2	Fast day.								
3*	" "								
4	Green veg. only containing			16	21.5	31.2	27.0	1.26	5.75
5	" " " "			40.5					
6	" " " "			60.0	29.2	42.3	44.1	1.51	6.51
7†	" " " "			11.5					
8	375	29.0	25.0	5.0					
9	630	50.0	43.0	"					
10	"	"	"	"					
11	"	"	"	"					
12	"	"	"	"	40.2	58.2	54.5	1.35	6.67
13	"	"	"	"					
14	655	51.5	43.5	10.0	32.4	47.0	50.2	1.55	6.70
15	170†	13.5	12.5						
16	Fast day.								
17	Green veg. only containing			10.0					
18	" " " "			20.0	32.0	46.4	51.9	1.62	6.61
19†	" " " "			12.5					
20	Fast day.								
21	355	24.5	23.5	10.0	27.3	39.6	51.2	1.88	6.50

* Drowsiness. † Partial fast.



1. Case 2481, female, age 12. Diabetic less than 3 months, mild, fair ability to burn carbohydrate (shown subsequently by a carbohydrate tolerance test).

The curves show a dangerous acidosis when the patient was placed on a fast. This was accompanied by clinical symptoms. The acidosis was ameliorated when a carbohydrate diet was instituted. The drop in alveolar CO₂ on the last day was evidently due merely to change in respiratory control.

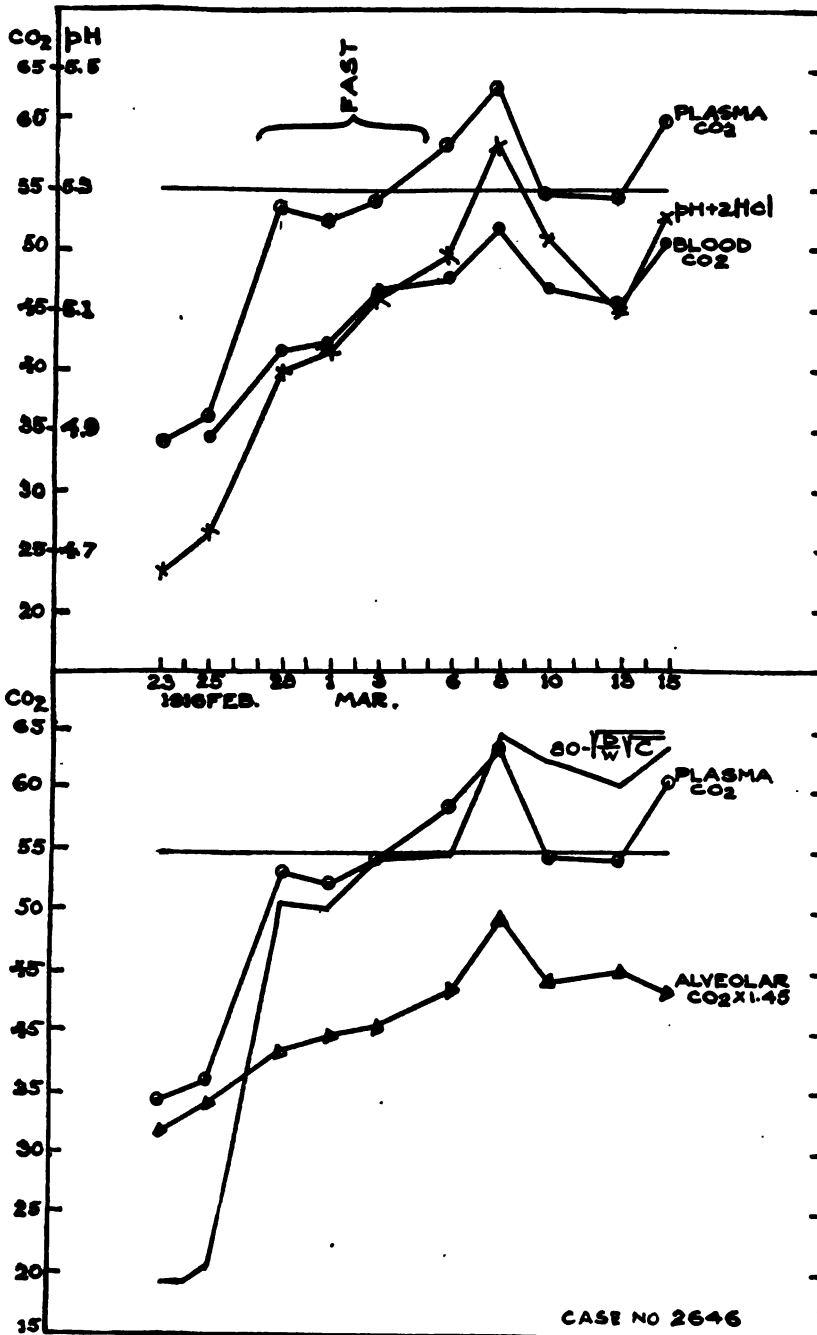
(From April 30-May 21, 1915.)

Group IV.

2. Case 2646.

Date.	Diet.				Body weight. kg.	Alveolar CO ₂ .		Blood.				Urine.					
	Total calories.	Protein. gm.	Fat. gm.	Carboby drate. gm.		mm.	mm. X 1.45	CO ₂ bound by 100 cc. plas- ma at 20°.	Ratio plasma CO ₂ mm. alv. CO ₂	CO ₂ bound by 100 cc. whole blood at 37°.	pH Plasma + 2 vol. N/50 HCl.	Vol. per 24 hrs. cc.	Urea per liter. cc. N/10 cc. N/10 cc. N/10	NH ₄ per liter. cc. N/10 cc. N/10 cc. N/10	Acid per liter. cc. N/10 cc. N/10 cc. N/10	$\sqrt{\frac{W}{D}}$	$80 - \sqrt{\frac{W}{D}}$
1916																	
Feb.																	
23*	2,000	75	138	100	37	22.2	31.9	34.3	1.54		4.67	2,600	1,650	1,175	224	60.6	19.4
24*	"	"	"	"	37.4							2,640	1,440	1,183	204	60.7	19.3
25*	"	"	127	125	37.6	23.5	34.1	36.0	1.53	34.7	4.73	2,560	1,318	1,205	174	59.5	20.5
26	"	"	"	"													
27	Fast day.				37.7	26.4	38.3	53.4	2.02	41.9	5.0	2,625	390	456	54	29.1	50.9
28	"																
29	"																
Mar.																	
1	"				37.2	27.3	39.6	52.1	1.91	42.1	5.03	1,950	650	568	74	29.7	50.3
2	"																
3	"				35.6	28.0	40.6	54.0	1.93	46.5	5.12	2,305	530	420	62	26.1	53.9
4	"																
5	Green veg. only containing			10													
6	"	"	"	20	35.6	30.0	43.5	58.9	1.97	47.4	5.19	2,140	754	423	64	25.2	54.8
7	"	"	"	"													
8	"	"	"	8.6	36.9	34.1	49.5	63.4	1.86	51.6	5.38	3,045	424	182	22	15.7	64.3
9	80	7.5	5.0														
10	160	15	10.5		36.3	30.5	44.2	54.9	1.80	46.8	5.22	2,600	598	232	28	17.3	62.7
11	320	30	21.0														
12	Fast day.																
13	Green veg. only containing			10.0	36.3	31.2	45.2	54.4	1.74	45.2	5.10	2,470	556	270	32	19.7	60.3
14	"	"	"	20.0													
15	"	"	"	30.0	35.8	29.8	43.2	60.9	2.04	50.9	5.26	3,010	416	164	60	16.5	63.5

* Abdominal cramps.



2. Case 2646, female, age 19. Diabetic of moderated severity for 4 years. There was a marked acidosis on admission (February 19, 1916).

The chart shows the curves, which had remained constant at a level of alarming acidosis for 7 days on a moderate mixed diet containing 100 gm. of carbohydrate, immediately rise to a low normal level on fasting without NaHCO_3 , gain a high level on a carbohydrate test, regress to a lower level again when a low carbohydrate-free diet was instituted, and again rise to normal when placed on another carbohydrate tolerance test. After recovery from acidosis the alveolar CO_2 is consistently much lower than it should be to indicate the blood bicarbonate.

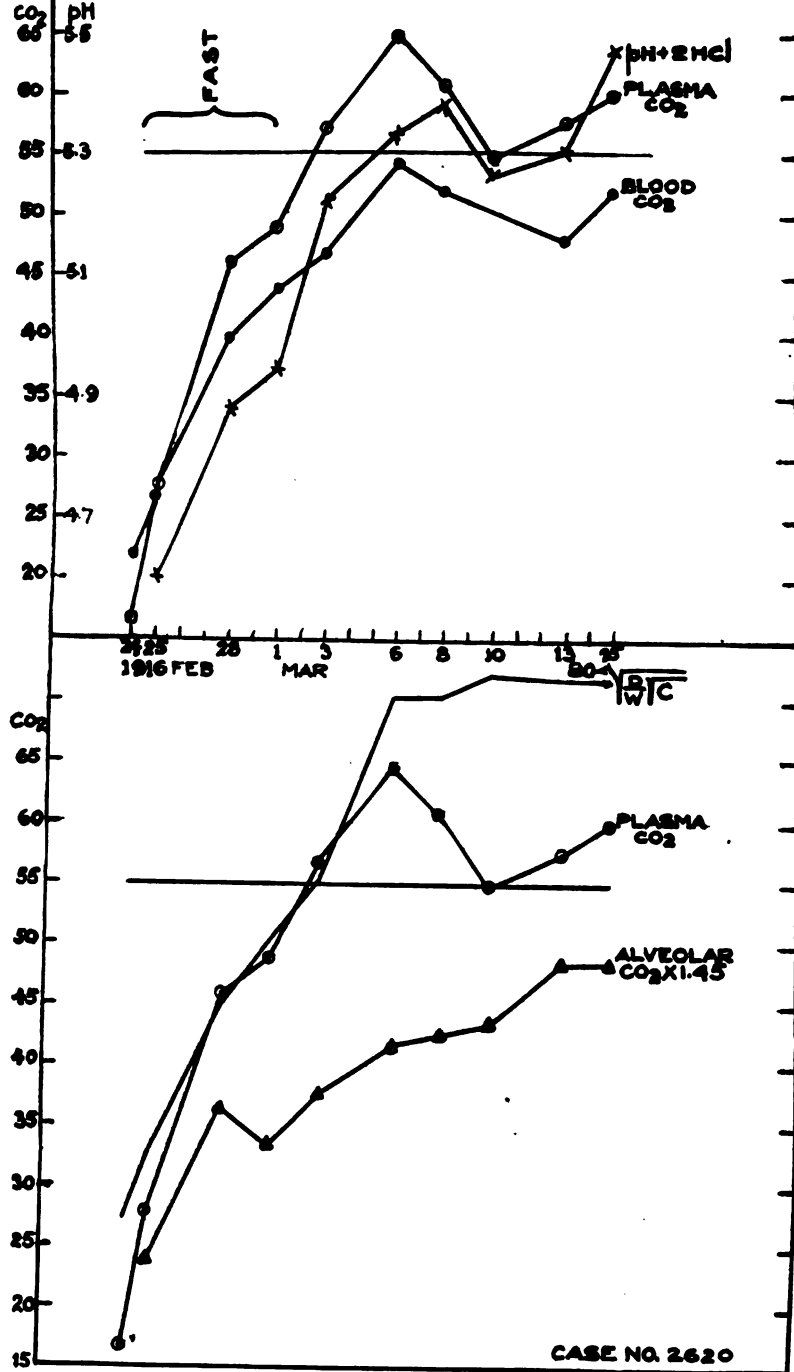
(From February 23-March 15, 1916.)

Group II

3. Case 2020.

Date.	Diet.				Body weight. kg.	Alveolar CO ₂ .		Blood.				Urine.					
	Total calories.	Protein.	Fat.	Carbohydrate.		mm.	mm. × 1.45	CO ₂ bound by 100 cc. plas- ma at 20°.	Ratio plasma CO ₂ mm. alv. CO ₂	CO ₂ bound by 100 cc. whole blood at 37°.	pH Plasma + 2 vol. HCl. N/50	Vol. per 24 hrs. cc.	Urea per liter. cc. N/10	NH ₄ per liter. cc. N/10	Acid per liter. cc. N/10	$\sqrt{\frac{W}{D}}$	$80 - \sqrt{D}$
1916																	
Feb.																	
24*	Fast day.				25			16.6		21.9		1,800	2,110	747	230	52.8	27.2
25	" "				25.6	16.3	23.6	27.7	1.70	27.5	4.61	2,905	1,165	580	144	47.1	32.9
26	" "																
27	" "																
28	" "				25.2	25.0	36.3	45.9	1.83	39.6	4.88	3,440	692	370	44	35.1	44.9
29	" "																
Mar.																	
1	Green veg. only containing			10	25.3	23.0	33.4	48.7	2.12	43.8	4.94	3,675	682	304	28	29.8	50.2
2	" "			20													
3	" "			30	26.4	25.9	37.6	56.9	2.20	46.5	5.22	3,300	532	260	20	24.8	55.2
4	" "			40													
5	" "			50													
6	" "			60	27.0	28.6	41.5	64.6	2.26	54.0	5.33	3,025	656	99	-12	9.7	70.3
7	" "			70													
8	" "			80	26.4	29.2	42.3	60.7	2.08	51.6	5.38	3,165	750	70	10	9.7	70.3
9	" "			90													
10	" "			100	25.8	29.8	43.2	54.5	1.83		5.26	3,260	772	57	4	7.9	72.1
11	" "			110													
12	" "			120													
13	" "			130	25.8	33.3	48.3	57.3	1.72	47.5	5.30	2,655	960	55	18	8.1	71.9
14	" "			140													
15	" "			150	25.8	33.3	48.3	59.9	1.80	51.9	5.48						

* Drowsiness; hyperpnea.



3. Case 2620, male, age 12. Diabetic 1 month, severe in that an extreme acidosis was present, as indicated both by laboratory and physical findings, but mild in carbohydrate tolerance (subsequently proven by a carbohydrate tolerance test).

On admission the curves indicated extreme acidosis. 6 days of fasting (with out NaHCO_3) obviated danger from coma. The subsequent 15 days of his carbohydrate tolerance test brought the acid curves to a normal level, at which they were maintained for the remainder of his stay in the hospital. The alveolar CO_2 after recovery from acidosis was consistently very much lower than the plasma CO_2 .

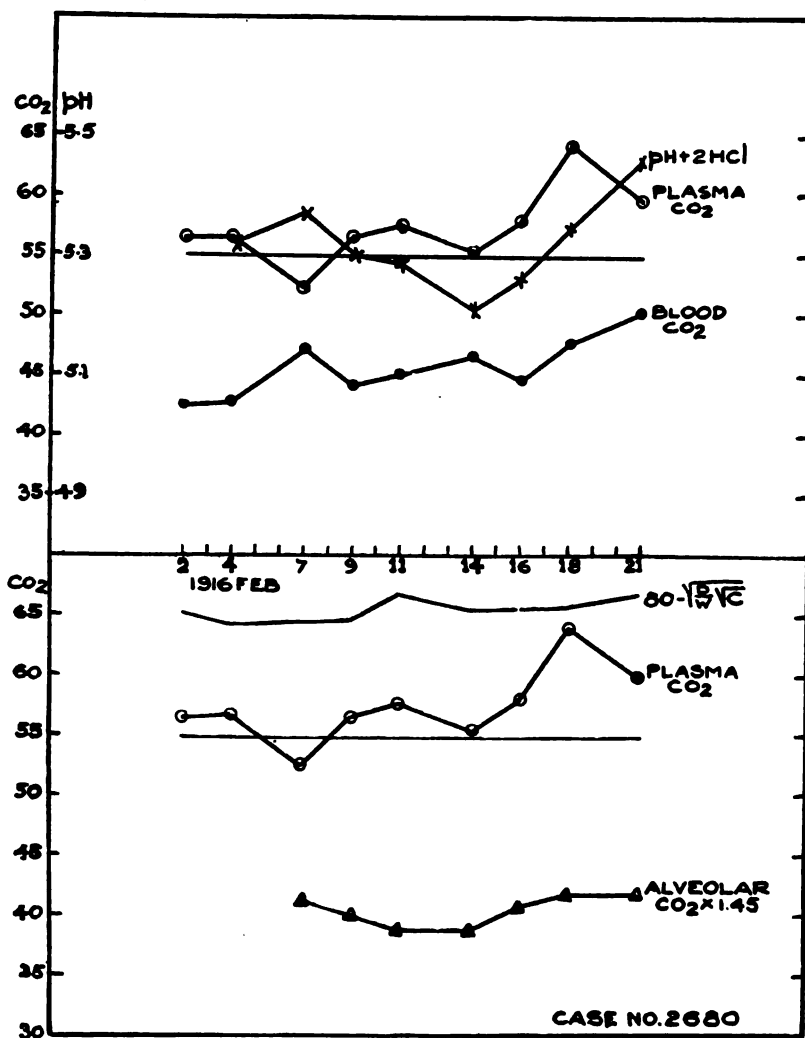
(From February 24–March 15, 1916.)

Group II.

4. Case 2680.

Date.	Diet.					Alveolar CO ₂ .		Blood.				Urine.				
	Total calories.	Protein.	Fat.	Carbohydrate.	Alcohol.			CO ₂ bound by 100 cc. plas- ma at 20°.	Ratio plasma CO ₂ mm. alv. CO ₂	CO ₂ bound by 100 cc. whole blood at 37°.	Plasma + 2 HCl. N/50	Vol. per 24 hrs.	Urea per liter.	NH ₃ per liter.	Acid per liter.	$\sqrt{\frac{W}{D}}$
		gm.	gm.	gm.	cc.	mm.	mm. X 1.45	cc.		cc.	ϕ H.	cc.	cc. N/10	cc. N/10	cc. N/10	$\sqrt{\frac{W}{D}}$
1916																
Feb.																
2	765	22.5	16	0	75	38.0		56.3		42.3		2,855	792	138	63	14.9
3	805	32.5	"	"	"			56.4		42.6	5.32	2,540	1,465	190	54	15.9
4	"	"	"	"	"	37.4										64.1
5	"	"	"	"	"											
6	525	Fast day.														
7	630	32.5	16		53	37.2	28.4	57.3	2.02	47.0	5.37	2,015	1,690	195	84	15.6
8	"	"	"	0	"											64.4
9	625	25.0	"	"	"	38.0	27.6	56.6	2.05	44.0	5.30	2,065	1,190	202	74	15.5
10	450*	7.5	5.5	"	"											64.5
11	370	Fast day.				37.7	26.8	67.8	2.16	45.0	5.29	2,425	1,285	147	46	13.1
12	450*	7.5	5.5	0	53											66.9
13	505	"	9.5	"	"											
14	530	15.0	10.5	"	"	38.0	26.8	55.2	2.06	46.6	5.21	2,710	925	158	52	14.7
15	610	22.5	16.0	"	"											65.3
16	440	15.0	10.5	"	40	37.9	28.1	58.0	2.06	44.5	5.26	2,380	1,250	214	58	16.7
17	"	"	"	"	"											63.3
18	320	4.0	2.5	"	"	37.6	28.8	64.1	2.22	47.9	5.35	2,730	925	167	36	14.4
19	360*	7.5	5.5	"	"											65.6
20	"	"	"	"	"											
21	400	11.0	8.0	"	"	37.2	28.8	59.9	2.08	50.1	5.46	2,865	902	147	34	13.3

* Partial fast.



4. Case 2680, female, age 29. Diabetic 10 months, of the extremest type, as shown by the inability to tolerate the 1,000 calories allowed although nearly half of the caloric intake was composed of alcohol. A moderate acidosis was manifested on admission (October 16, 1915).

The curves (taken during the 4th month of hospital observation) show a tendency to acidosis by continually approaching the lower border of normal. Possibly this is the result of the necessarily low diet. The alveolar CO₂ is much too low to show the true blood bicarbonate.

(From February 2-21, 1916.)

Group III.

5. Case 2686.

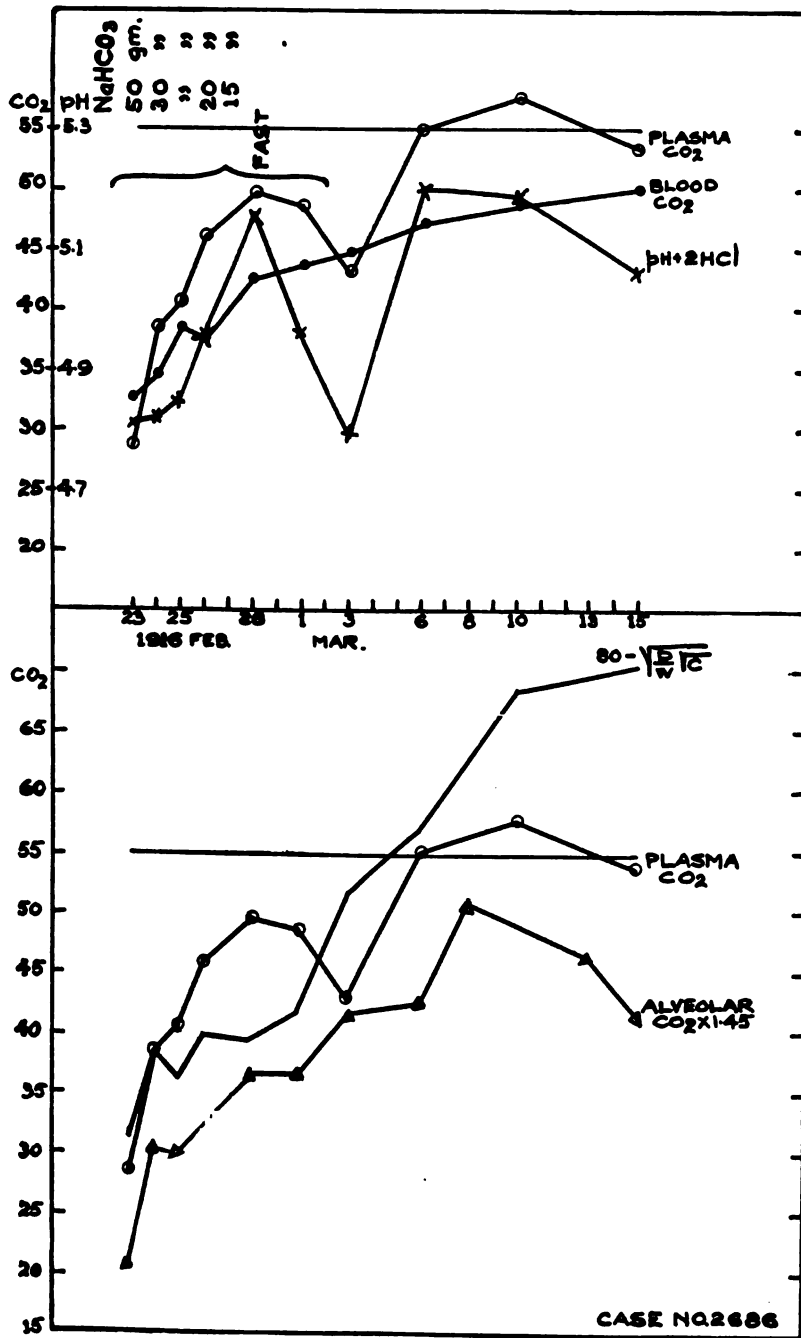
Date.	Diet.				Body weight.	Alveolar CO ₂ .				Blood.				Urine.				
	Total calories.	Protein.	Fat.	Carbohydrate.		NaHCO ₃ .	Alveolar CO ₂ .		CO ₂ bound by 100 cc. plasma at 20°.	Ratio plasma CO ₂ mm. alv. CO ₂	CO ₂ bound by 100 cc. whole blood at 37°.	Plasma + 2 vol. n/50 HCl.	Vol. per 24 hrs.	Urea per liter.	NH ₃ per liter.	Acid per liter.	$\sqrt{\frac{W}{D} \cdot \frac{W}{C}}$	$80 - \sqrt{\frac{W}{D} \cdot \frac{W}{C}}$
							gm.	mm. X 1.45										
1916																		
Feb.																		
22*	Fast day.				45	27.8	14.0	20.3	28.6	2.04	32.7	4.81	6,530	915	310	128	48.7	31.3
23**	"				50	26.0	20.8	30.2	38.3	1.84	33.5	4.82	4,915	732	317	128	42.6	38.4
24†	"				30	25.4	20.6	29.9	40.4	1.96	38.3	4.85	4,760	718	362	112	43.9	36.1
25†	"				30	25.0			46.0		37.8	4.97	3,115	888	481	92	40.1	39.9
26	"				20													
27	"				15													
28	"					25.4	25.3	36.7	49.6	1.96	42.8	5.16	3,335	575	483	60	40.7	39.3
29	"																	
Mar.																		
1	"					25.0	25.2	36.5	48.7	1.93	43.8	4.96	3,685	482	336	36	38.5	41.5
2	Green veg. only containing			10		25.6	20.88	41.8	43.0	1.49	44.7	4.79	1,635	862	526	38	28.3	51.7
3	"			20														
4	"			"														
5	"			"	25													
6	"			"	"	30.0	29.2	42.3	55.0	1.89	47.0	5.20	2,330	464	342	20	23.1	56.9
7	"			"	"													
8	"			"	30	31.8	28.5						2,965	436	185	14	16.5	63.5
9	"			"	35													
10†	"			"	8.8	31.6	35.5	50.8	57.6	1.62	48.5	5.19	2,820	502	113	60	11.7	68.3
11	Fast day.																	
12	Green veg. only containing			5														
13	"			10		29.8	31.9	46.3								18	9.8	70.2
14	"			20														
15	"			"		29.4	28.4	41.2	53.6	1.88	50.0	5.06	3,520	332	69			

* Drowsiness; hyperpnea; nausea; practically moribund.

** Drowsiness; hyperpnea.

† Hyperpnea.

‡ Partial fast.



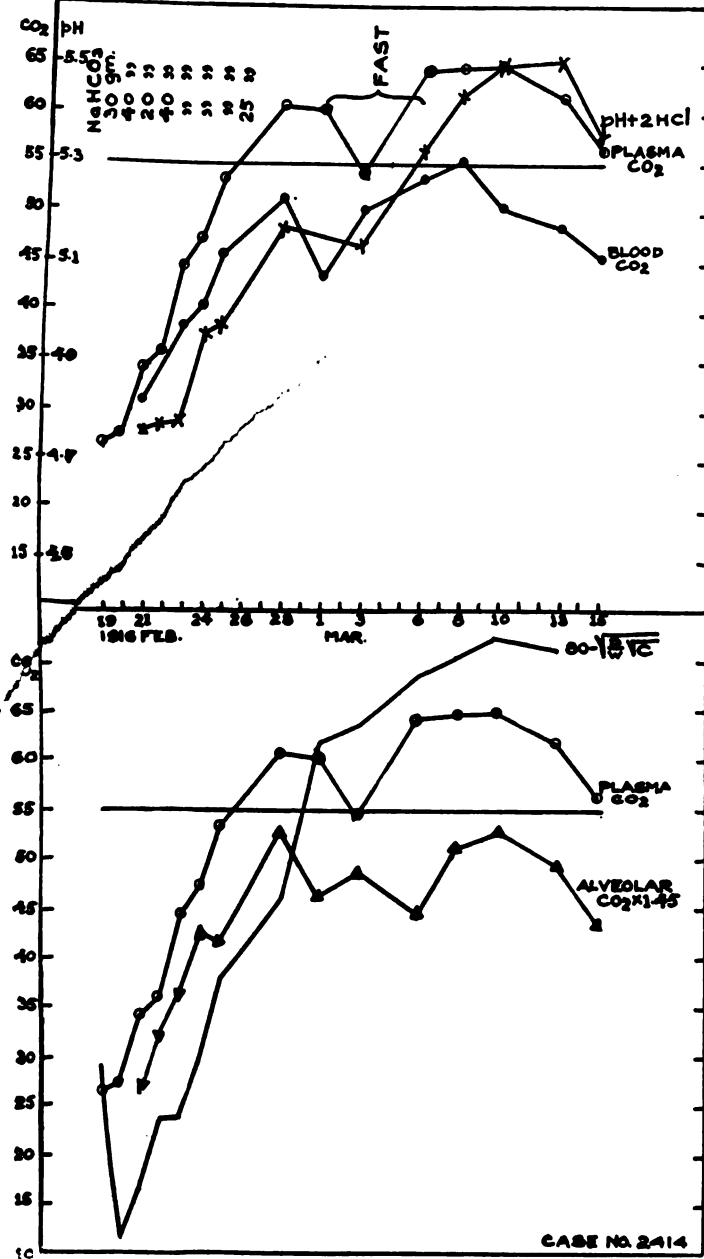
5. Case 2686, male, age 13. Diabetic 1 year, extreme in acid intoxication, rather severe in carbohydrate tolerance (subsequently shown by a carbohydrate tolerance test).

The curves indicate an acidosis bordering on coma, rapidly coming out of danger through fasting with NaHCO₃ therapy, but maintaining a subnormal level throughout. It might be prognosed from these curves, in such a young case, that the course of the disease, even under a strict régime, would be progressive. The alveolar CO₂ is consistently lower than the plasma bicarbonate. On March 3 the plasma bicarbonate and hydrogen ion concentration show a fall which is presumably due to exposure of the blood sample to CO₂ before centrifugation since the whole blood figure as well as the acid excretion and alveolar CO₂ show no drop on this date. The absence of this phenomenon in the curves of other

6. Case 2414.

Date.	Diet.				Body weight.	Alveolar CO ₂ .		Blood.				Urine.						
	Total calories.	Protein.	Fat.	Carbohydrate.		NaHCO ₃ .	mm.		CO ₂ bound by 100 cc. plasma at 20°.	Ratio plasma CO ₂ to mm. alv. CO ₂	CO ₂ bound by 100 cc. whole blood at 37°.	Plasma + 2 vol. HCl. N/50.	Vol. per 24 hrs.	Urea per liter.	NH ₃ per liter.	Acid per liter.	$\sqrt{\frac{W}{D} \times C}$	$80 - \sqrt{\frac{W}{D} \times C}$
							gm.	gm.										
1916																		
Feb.																		
19*	845	162.5	19	0	30	44.6		26.4					1,130	3,470	1,110	544	50.8	29.2
20	600	94.	23	"	40	44.9		24.2					3,775	3,480	1,102	352	68.56	11.4
21	"	"	"	"	20	44.8	18.0	34.3	1.90	31.0	4.86		3,980	4,010	1,025	252	63.6	16.4
22	"	"	"	"	40	44.6	22.3	36.0	1.61		4.87		3,620	3,885	1,065	264	61.4	18.6
23	400	63.	15	"	"	44.6	25.0	44.5	1.78	38.3	4.88		4,005	3,910	955	248	61.4	18.6
24	"	"	"	"	"	45.0	29.4	47.2	1.61	40.4	4.96		3,395	3,685	833	208	55.0	29.5
25	"	"	"	"	"	46.0	28.4	53.3	1.88	45.6	4.98		3,375	3,040	624	200	42.0	38.0
26	200	31.5	7.5	"	25			65.5										
27	"	"	"	"	"													
28	"	"	"	"	"	18.9	36.4	60.9	1.67	51.3	5.18		5,610	2,080	339	122	34.1	45.9
Mar.																		
1	Fast day.							60.2	1.90	43.3			5,080	1,240	175	60	19.3	60.7
2	"					48.0	31.7											
3	"					47.4	33.8	54.0	1.60	50.3	5.14		3,450	1,235	179	60	16.9	63.1
4	"																	
5	"																	
6	Green veg. only containing			10		47.0	37.0	44.5	1.73	53.1	5.33		3,290	1,060	117	32	11.4	68.6
7	"	"	"	10														
8	"	"	"	15		47.2	35.5	51.5	1.81	55.1	5.44		3,500	1,140	100	18	9.7	70.3
9	"	"	"	20														
10	"	"	"	25		46.2	35.5	51.5	1.82	50.5	5.50		3,175	895	830	8	7.6	72.4
11	"	"	"	30														
12	"	"	"	3.3														
13	160	15	10	0		14.4	34.1	49.5	1.81	48.9	5.51		3,315	1,095	78	24	8.8	71.2
14	240	22.5	16	0														
15	Green veg. only containing			10.0		44.4	29.8	43.2	1.89	45.2	5.34		3,275	1,042	97	24	10.0	70.0

* Drowsiness; hyperpnea; nausea.



6. Case 2414, male, age 17. Diabetic 1 year of a rapidly progressive type. Extreme acidosis on his first admission (March 19, 1915), responded to the fast-ing treatment, and again on the present admission. On this occasion the acidosis was precipitated by a self-directed fast of 7 days.

The chart shows the curves, in a patient brought to the border of coma by ill-advised fasting, respond to treatment consisting of a low protein-fat (carbo-hydrate-free) diet, sodium bicarbonate in moderate dosage, and forcing of fluid. After recovery from this extreme acidosis, the alveolar CO_2 remained consistently subnormal. The acid excretion during NaHCO_3 feeding is, as usual, greater than corresponds to the degree of internal acidosis. The discrepancy disappears the day after NaHCO_3 feeding ended.

(From February 19-March 15, 1916.)

Group IV.

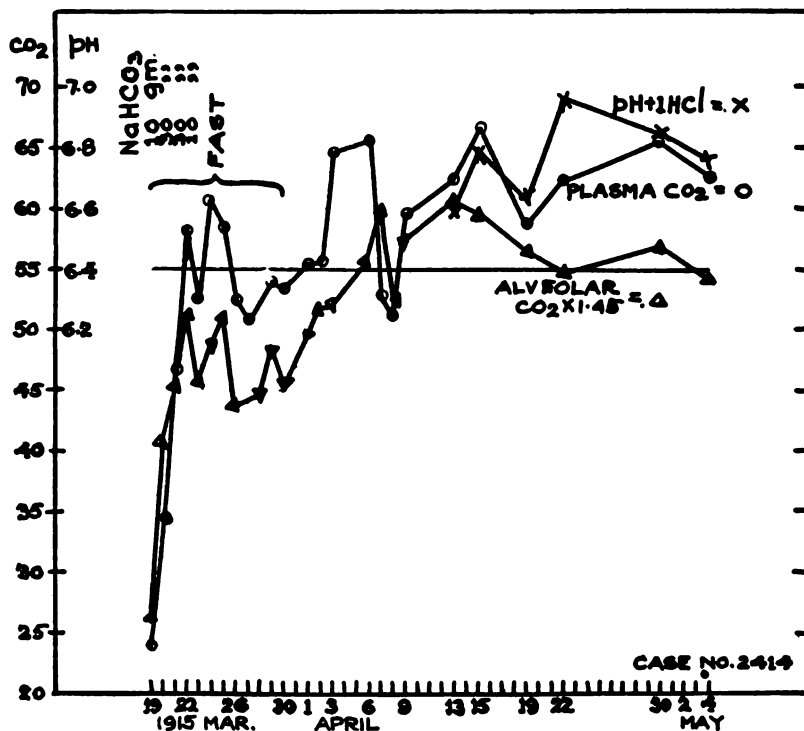
7. Case 2424.

Date.	Total calories.	Protein.	Fat.	Carbohydrate.	NaHCO ₃ .	Alcohol.	Alveolar CO ₂ .		CO ₂ bound by 100 cc. plasma at 20°.	Ratio CO ₂ plasma CO ₂ min. alv. CO ₂ .	Plasma + HCl.
1915	gm.	gm.	gm.	gm.	gm.	cc.	mm.	mm. X 1.45	cc.		pH
Mar. 19*	245 Fast day with whiskey.				10	35	17.9	26.0	23.8	1.33	
20†	560	"	"	"	30	80	28.0	40.6	34.1	1.22	
21	"	"	"	"	30	80	31.5	45.7	46.8	1.49	
22	525	"	"	"	10	75	35.2	51.1	58.5	1.66	
23	560	"	"	"		80	31.5	45.7	52.4	1.66	
24	385	"	"	"		55	33.7	48.9	60.6	1.80	
25	"	"	"	"			35.4	51.4	58.0	1.64	
26	"	"	"	"			30.2	43.8	52.4	1.73	
27	"	"	"	"					50.4		
28	"	"	"	"			30.6	44.4			
29	"	"	"	"			33.3	48.3	54.8	1.62	
30	Green veg. only containing						31.1	45.1	53.4	1.72	
31	"	"	"	"	10						
Apr. 1	"	"	"	"	20		34.2	49.6	55.2	1.62	
2	"	"	"	"	"		35.9	52.0	55.2	1.54	
3	"	"	"	"	30		35.9	52.0	64.8	1.80	
4	"	"	"	"	50						
5	"	"	"	"	70						
6	"	"	"	"	80		38.5	55.8	65.6	1.70	
7	"	"	"	"	90		41.4	60.0	52.9	1.28	
8	"	"	"	"	100		35.4	51.4	51.4	1.45	
9	"	"	"	"	150		39.4	57.2	59.5	1.51	
10	"	"	"	"	"						
11	"	"	"	"	"						
12	"	"	"	"	175						
13	"	"	"	"	"		41.7	60.5	62.1	1.49	6.60
14	"	"	"	"	"						
15	"	"	"	"	200		41.0	59.5	66.6	1.62	8.80
16	Fast day.										
17	765	33	56	25							
18	1,240	48	100	"							
19	1,670	"	147.5	"			38.7	56.1	58.5	1.51	6.63
20	"	"	"	"							
21	"	"	"	"							
22	"	"	"	"			37.8	54.8	62.1	1.64	6.97
23	"	"	"	"							
24	1,700	50	149.5	"							

* Drowsiness: hyperpnea.

† Drowsiness.

Date.	Total calories.	Protein.	Fat.	Carbohydrate.	NaHCO ₃ .	Alcohol.	Alveolar CO ₂ .		CO ₂ bound by 100 cc. plasma at 20°.	Ratio plasma CO ₂ mm. alv. CO ₂ .	Plasma + 1 vol. N/50 HCl.
1915		gm.	gm.	gm.	gm.	cc.	mm.	mm. X 1.45	cc.		pH
Apr. 25	Fast day.										
26	1,835	53	156.5	40							
27	1,845	55	"	"							
28	"	"	"	"							
29	"	"	"	"							
30	1,840	52.5	"	"			39.3	57.0	65.5	1.67	6.86
May 1	"	"	"	"							
2	Fast day.										
3	1,840	52.5	156.5	40							
4	1,985	75.5	162.5	"			37.2	54.0	62.3	1.67	6.77



7. Case 2414, male, age 16. Diabetic less than 1 month. Mild intolerance for carbohydrate (proven by subsequent carbohydrate tolerance test) but extreme acid intoxication, the patient being on the verge of coma on admission (March 19, 1915).

The curves are shown to illustrate the response of a case, on the border of coma to the fasting treatment with moderate doses of NaHCO₃. On this occasion the patient responded to such treatment. Chart 6 shows the diametrically opposite result obtained by fasting. These two charts taken together illustrate the necessity of having a patient under careful observation during the fasting period, and the impossibility of predicting the behavior even of a patient who has been fasted before.

(From March 19–May 4, 1915.)

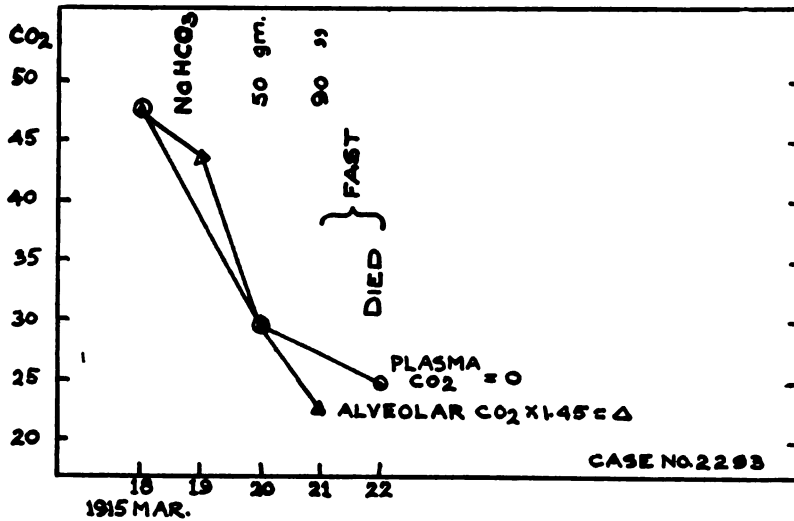
Group II.

8. Case 2293.

Date.	Total calories.	Protein.	Fat.	NaHCO ₃ .	Alcohol.	Alveolar CO ₂ .		CO ₂ bound by 100 cc. plas- ma at 20°.	Ratio plasma CO ₂ mm. alv. CO ₂	Plasma + 1 cc. 10% HCl.
1915		gm.	gm.	gm.	cc.	mm.	mm. × 1.45	cc.		pH
Mar.										
18	1,285	41.0	71.5		65	32.4	47.0	47.3	1.46	
19	1,815	62.5	115.0		70	30.0	43.5			
20*	1,635	53.0	123.0	50	25	20.5	29.7	25.9	1.26	
21*†	Fast day.			90	35	15.8	22.9	24.9 (Death.)		
22*†	" "									

* Drowsiness; hyperpnea; nausea.

† Moribund.



8. Case 2293, male, age 29. Diabetic 2 years, of the severest type in intolerance of any food. Complicated at the end by pulmonary tuberculosis.

The curves show failure of response to large doses of NaHCO₃. Death occurred March 22, 1915.

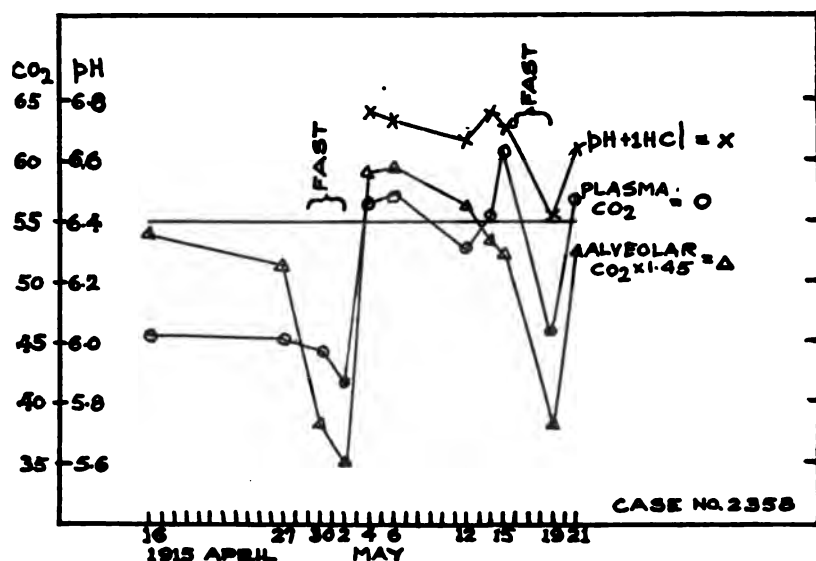
(From March 18-22, 1915.)

Group III-IV.

9. Case 2358.

Date.	Total calories.	Protein.	Fat.	Carbohydrate.	Alveolar CO ₂ .		CO ₂ bound by 100 cc. plasma at 20°.	Ratio plasma CO ₂ mm. alv. CO ₂	Plasma + ¹ / _N 50 HCl.
1915		gm.	gm.	gm.	mm.	mm. × 1.45	cc.		pH
Apr. 16	1,915	62.5	178.5	0	37.2	54.0	45.8	1.23	
" 17	"	"	"	"	"	"	"	"	"
18	Fast day.								
19	1,325	49.0	118.5	5					
20	"	"	"	"					
21	"	"	"	"					
22	"	"	"	"					
23	"	"	"	"					
24	"	"	"	"					
25	Fast day.								
26	1,350	50.0	119.0	10					
27	"	"	"	"	35.4	51.5	45.3	1.28	
28	325*	21.0	26.0						
29	Fast day.								
30	"	"	"		26.5	38.4	44.1	1.61	
May 1	"	"	"						
2	1,020	29.0	92.5	10	24.1	35.0	41.6	1.73	
3	1,350	50.0	119.0	"					
4	"	"	"	"	40.8	59.2	56.8	1.39	6.76
5	"	"	"	"					
6	1,375	52.0	"	15	40.7	59.0	57.0	1.40	6.73
7	1,495	52.5	131.5	"					
8	"	"	"	"					
9	Fast day.								
10	1,535	54.0	132.0	20					
11	"	"	"	"					
12	"	"	"	"	38.8	56.3	52.7	1.36	6.67
13	"	"	"	"					
14	1,595	57.0	133.0	30	36.8	53.4	55.2	1.50	6.76
15	250*	13.5	21.0		36.1	52.4	60.8	1.68	6.72
16	Fast day.								
17	"	"	"						
18	"	"	"						
19	715*	25.0	50.0	10	26.2	38.0	45.9	1.69	6.41
20	1,035	60.5	80.5	"					
21	"	"	"	"	36.0	52.2	56.9	1.58	6.64

* Partial fast.



9. Case 2358, age 11. Diabetic 1 year, of severe type in intolerance of any food.

The curves show a sudden development of acidosis on two separate fasting periods of 3 days each with a return to normal on the resumption of diet.

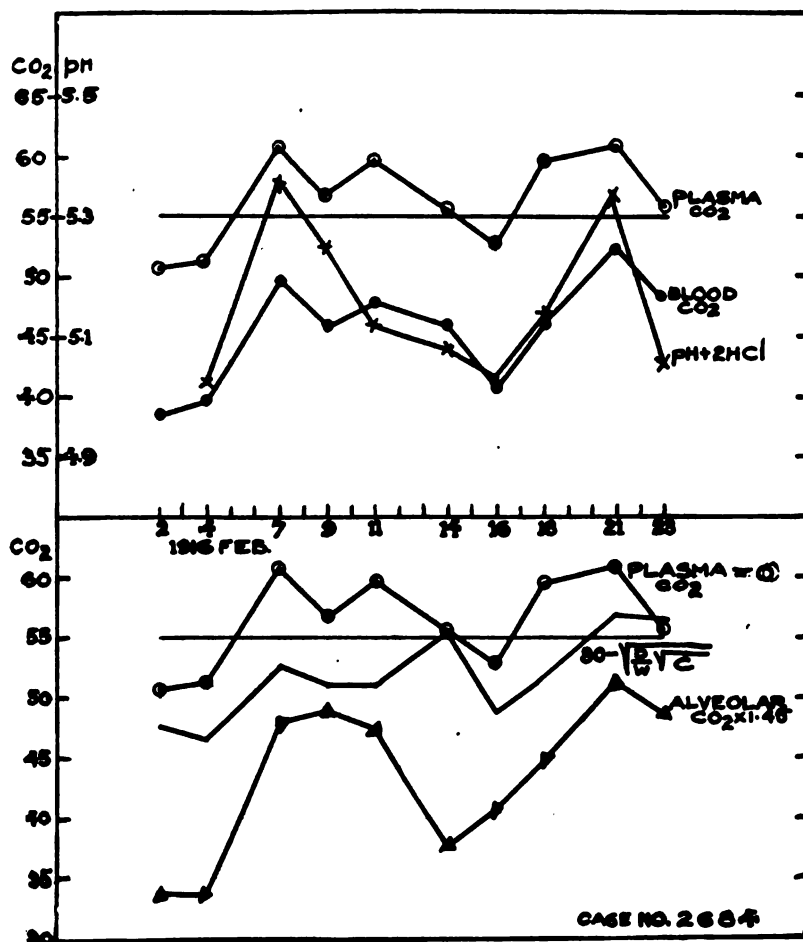
(From April 16-May 21, 1915.)

Group III.

10. Case 2684.

Diet.					Blood.					Urine.									
Date.	Total calories.	Protein.	Fat.	Carbohydrate.	Alcohol.	Body weight.	Alveolar CO ₂ .		CO ₂ bound by 100 cc. plasma at 20°.	Ratio plasma CO ₂ mm. alv. CO ₂	CO ₂ bound by 100 cc. whole blood at 37°.	Plasma + 2 HCl. vol. N/50	Vol. per 24 hrs.	Urea per liter.	NH ₃ per liter.	Acid per liter.	$\sqrt{\frac{W}{D \cdot C}}$	$80 - \sqrt{\frac{W}{D \cdot C}}$	
							mm.	mm. X 1.45											
1916																			
Feb.																			
2	855	35	27.5	0	65	34.8			50.8		38.1		2,220	918	507	180	32.4	47.6	
3	860	40	33.5	"	55														
4	855	45	38.5	"	45	35.3	23.1	33.5	51.1	2.21	39.7	5.01	1,790	1,425	662	152	33.5	46.5	
5	850	50	43.0	"	35														
6	525	Fast day.				75													
7	850	40	40.0	"	45	35.5	33.0	47.9	60.6	1.84	49.3	5.36	1,665	1,580	532	116	27.2	52.8	
8	"	"	"	"	"														
9	695	35	27.5	"	"	37.0	33.5	48.6	56.7	1.69	45.5	5.25	1,780	1,570	539	136	29.0	51.0	
10	450*	12.5	9.5	"	"														
11	315	Fast day.				"	32.7	47.4	59.7	1.83	47.8	5.12	2,070	1,535	534	72	29.0	51.0	
12	470*	14.5	10.5	"	"														
13	555	22.5	16.0	"	"														
14	575	27.5	"	"	"	37.0	26.1	37.8	55.2	2.11	45.6	5.08	2,280	964	365	90	24.5	55.5	
15	605	32.5	16.5	"	"														
16	625	37.5	"	"	"	37.3	28.1	40.8	52.5	1.83	40.4	5.03	1,855	1,465	612	108	31.1	48.9	
17	650	42.5	17.5	"	"														
18	"	"	"	"	"	38.0	30.9	44.8	59.3	1.92	46.0	5.13	2,345	1,225	445	70	28.3	51.7	
19	"	"	"	"	"														
20	315	Fast day.				"													
21	650	42.5	17.5	"	"	37.2	35.3	51.2	60.9	1.72	52.1	5.34	1,545	1,408	470	84	23.0	57.0	
22	680	35.0	24.0	"	"														
23	710	40.0	"	"	"	38.0	33.6	48.7	55.6	1.65	47.3	5.05	1,965	1,415	399	96	24.0	56.0	

* Partial fast.



10. Case 2684, female, age 43.

The curves show a continuous low grade acidosis, possibly caused by a very low protein-fat (carbohydrate-free) diet, necessitated by a persistent glycosuria. The low level of these curves was subsequently noted over a period of 4 months' observation. The acid excretion corresponds to the plasma bicarbonate, but the alveolar CO_2 was consistently very much too low.

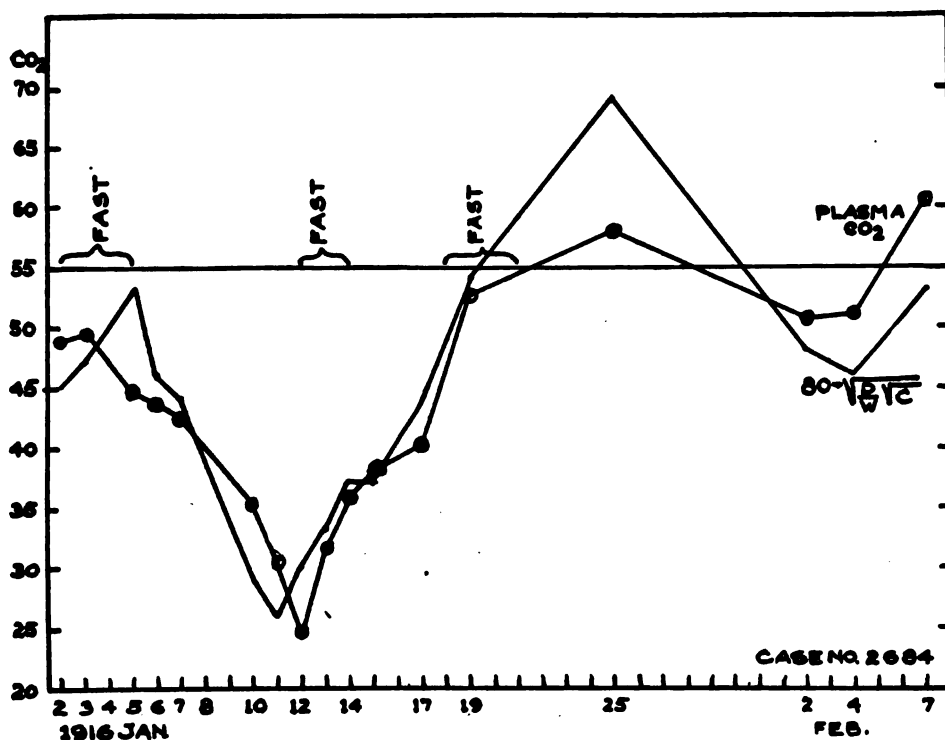
(From February 2-23, 1916.)

Group III.

11. Case 2684.

Date.	Diet.					Body weight. kg.	Blood. CO ₂ bound by 100 cc. plasma at 20°.	Urine.				
	Total calories.	Protein. gm.	Fat. gm.	Carbohydrate. gm.	Alcohol. cc.			Vol. per 24 hrs. cc.	Urea per liter. cc. N/10	NH ₃ per liter. cc. N/10	Acid per liter. cc. N/10	$\sqrt{\frac{W}{D}}$
1916												$80 - \sqrt{\frac{W}{D}}$
Jan. 2	Fast day.					36.0	47.8	2,150	2,120	530	230	35.0
3	" "					35.3	48.2	1,615	1,475	579	250	33.0
4	" "											45.0
5	160	15	10.5	0		35.0	44.5	2,170	1,352	350	165	27.0
6	320	30	21.0	"		34.2	43.8	1,830	2,010	602	195	34.0
7	1,075	44.5	52.0	"	60	34.7	42.5	2,285	2,283	596	165	36.0
8	1,525	60.0	92.5	"	"							44.0
9	"	"	"	"	"							
10	2,145	74.5	153.0	"	"	34.1	35.2	1,975	2,375	942	325	29.0
11	1,830	"	"	"	15	34.3	30.4	2,120	2,695	964	345	26.0
12*	Fast day.					34.7	24.6	3,355	998	678	200	30.0
13	" "					33.6	31.6	2,720	785	701	185	33.0
14	160	15	10.5	0		33.3	35.9	2,300	1,010	709	148	37.0
15	240	22.5	16.0	"		33.3	38.1	2,210	1,358	734	180	37.0
16	Fast Day.											
17	500*	7.5	5.5	0	60	33.2	40.0	2,520	848	574	105	44.0
18	420	Fast Day.										
19	"	"			"	33.2	52.8	1,440	1,550	540	85	54.0
20	"	"			"							
21	420	Green veg. only containing		10	"							
22	525	"	"	20	75							
23	"	"	"	"	"							
24	"	"	"	25	"							
25	"	"	"	30	"	33.6	58.0	2,155	850	98	60	69.0

* Drawalinea; hypertonia; nausea.



11. Case 2684, female, age 43 $\frac{1}{2}$ (the same case as No. 10). Diabetic of extreme severity with marked emaciation. A mild acidosis was present on admission (January 1, 1916).

The curves show a dangerous response to a moderate protein-fat (carbohydrate-free) diet, which was accompanied by clinical manifestations of severe acidosis. At the end of 9 days' fasting the patient may be considered past danger from an acidosis point of view, as is indicated by the rise of the curves. The curves show consistent parallelism between acid excretion and plasma bicarbonate.

(From January 2-25, 1916.)

Group II.

12. Case 2128.

Date.	Diet.					Body weight.	Blood.		Urine.				
	Total calories.	Protein.	Fat.	Carbohydrate.	NaHCO ₃ .		CO ₂ bound by 100 cc. plas- ma at 20°.	CO ₂ bound by 100 cc. whole blood at 37°.	Vol. per 24 hrs.	Urea per liter.	NH ₃ per liter.	Acid per liter.	$\sqrt{\frac{W}{D}}$
1916 Mar. 20	625	40	47.5	7	30	37.0	33.3	29.9	7,740	390	312	102	
21*	Fast day.					37.0	60.1	48.4	4,400	456	315	48	42.0
22	" "					36.9	45.9	38.8	3,995	442	388	68	28.7
23	" "					37.0							33.4
24	120†	20	3.5			36.6	48.2	38.4	3,635	524	385	40	29.4
25	800	40	68.5			36.8	51.5	45.0	3,210	840	431	50	30.3
26	655	50	48.5										
27	600	70	33.5			37.5	50.2	42.4	3,590	1,225	412	76	31.9
28	Green veg. only containing			10		38.0	52.6	43.5	4,980	500	232	38	24.5
29	" " " "			20		37.7	58.3	46.7	4,740	454	200	36	21.2
30	" " " "			30									
31	" " " "			40		36.4	63.6	52.0	5,145	400	94	24	13.3

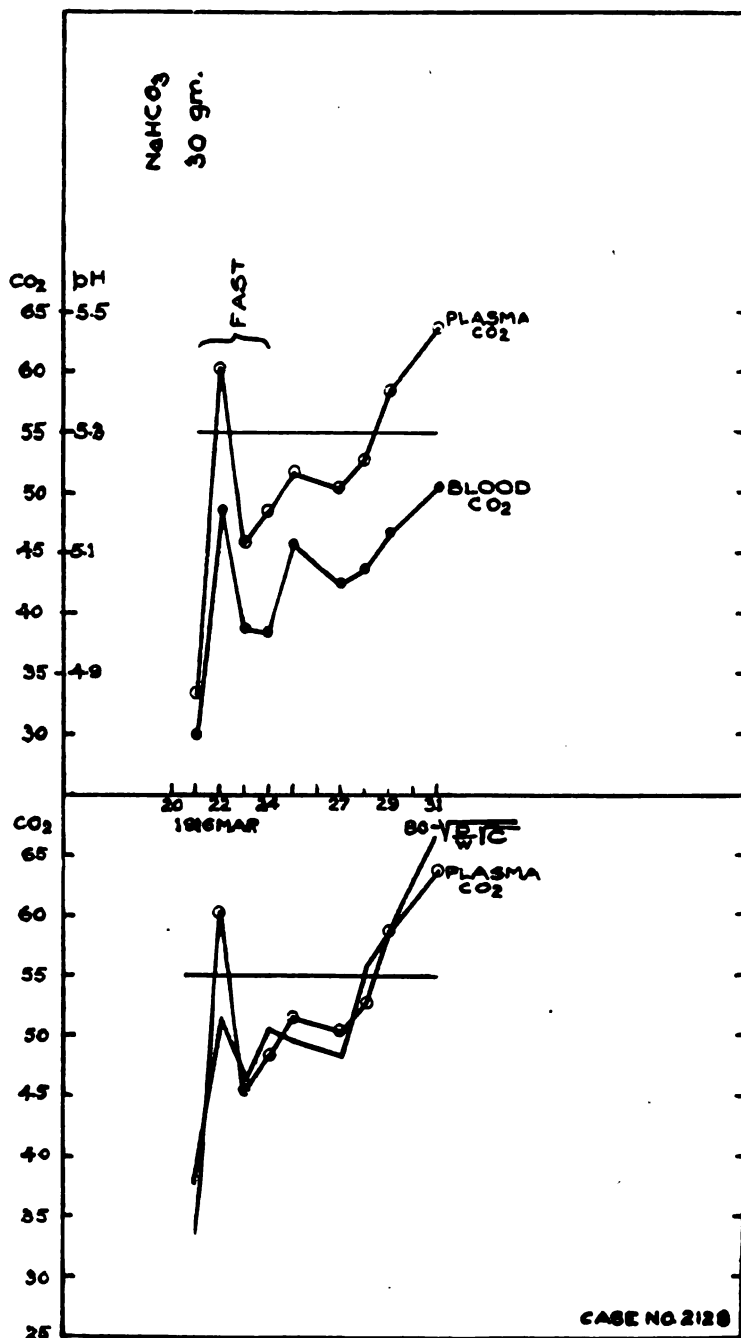
* Drowsiness.

† Partial fast.

12. Case 2128, female, age 19. Diabetic 4 years, of extreme severity in the inability to metabolize carbohydrate or fat, but without marked emaciation or loss of strength. Over 2 years of observation has shown a constant low grade acidosis.

The curves show the dangerous effect of the unrestricted diet indulged in prior to her readmission, with the striking result gained by fasting and a carbohydrate tolerance test. The parallelism between acid excretion and plasma bicarbonate was also striking. (From March 20-31, 1916.)

Group II.



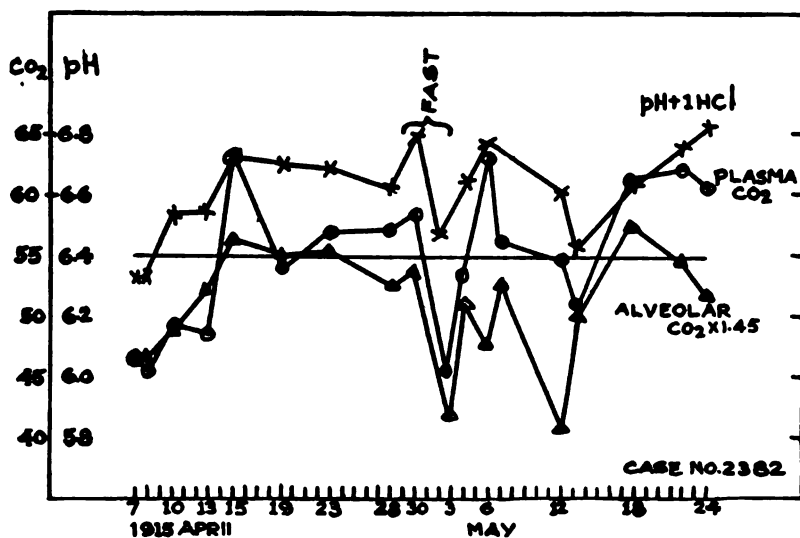
13. Case 2382.

Date.	Total calories.	Protein.	Fat.	Carbohydrate.	Alcohol.	Alveolar CO ₂ .		CO ₂ bound by 100 cc. plasma at 20°.	Ratio CO ₂ plasma alv. CO ₂	Plasma + 1 vol. N/50 HCl.
	gm.	gm.	gm.	cc.	mm.	mm. × 1.45	cc.		pH	
1915										
Apr. 7	Green veg. only containing			15.0	15	32.0	46.4	46.3	1.45	6.33
8	"	"	"	20.0		32.2	46.7	45.3	1.41	6.32
9	"	"	"	30.0						
10	"	"	"	49.0		33.5	48.6	49.0	1.46	6.55
11*	"	"	"	19.0						
12	2,440	85.5	224.5							
13	2,440	92.5	222.0			35.9	52.1	48.4	1.35	6.55
14	2,500	97.0	226.5							
15	2,440	88.0	224.0			38.8	56.2	63.1	1.63	6.73
16	2,440	92.5	121.5							
17	2,440	85.5	224.5							
18	Fast day.									
19	2,440	92.5	221.5			38.0	55.1	54.0	1.42	6.70
20	2,440	92.5	221.5							
21	2,440	85.5	224.5							
22	2,420	93.0	219.5							
23	2,440	85.5	224.5			38.3	55.6	56.0	1.46	6.68
24	2,440	92.5	221.5							
25	Fast day.									
26	2,210	92.5	196.5							
27	2,210	92.5	196.5							
28	2,185	93.0	194.5			36.4	52.9	56.0	1.53	6.62
29	Fast day.									
30	"	"				37.0	53.7	57.3	1.55	6.80
May 1	"	"								
2	"	"								
3	Green veg. only containing			20.0		28.7	41.6	45.1	1.57	6.46
4	"	"	"	40.0		35.2	51.0	53.2	1.52	6.64
5	"	"	"	50.0						
6	"	"	"	60.0		32.7	47.4	63.0	1.93	6.75
7	"	"	"	70.0		36.4	52.8	56.0	1.54	
8	"	"	"	80.0						
9	"	"	"	90.0						
10	"	"	"	100.0						
11*	"	"	"	24.5						

13. Case 2382—Concluded.

Date.	Total calories.	Protein.	Fat.	Carbohydrate.	Alcohol.	Alveolar CO ₂ .		CO ₂ bound by 100 cc. plasma at 20°.	Ratio plasma CO ₂ mm. alv. CO ₂	Plasma + 1 vol. x/50 HCl.
		gm.	gm.	gm.	cc.	mm.	mm. X 1.45	cc.		pH
1915										
May										
12	555	24.0	44.5	10.0		27.8	40.3	54.5	1.96	6.61
13	930	43.0	72.0	20.0		34.4	49.9	50.9	1.48	6.42
14	1,230	47.0	105.5	15.0						
15	1,370	62.0	113.5	15.0						
16	555*	21.5	49.0	3.0						
17	935*	46.5	80.0	12.5						
18	1,530	78.0	123.0	15.0		39.3	57.3	61.3	1.56	6.64
19	1,520	60.5	130.5	15.0						
20	1,450	70.0	123.5	3.5						
21	1,430	71.0	122.5							
22	1,425	60.5	127.0			37.7	54.7	62.3	1.65	6.76
23	Fast day.									
24	1,425	53.0	130.0			35.6	51.6	60.6	1.70	6.83

* Partial fast.



13. Case 2382, male, age 40. Diabetic 8 years, severe in intolerance of food, with a tendency to a low grade chronic acidosis.

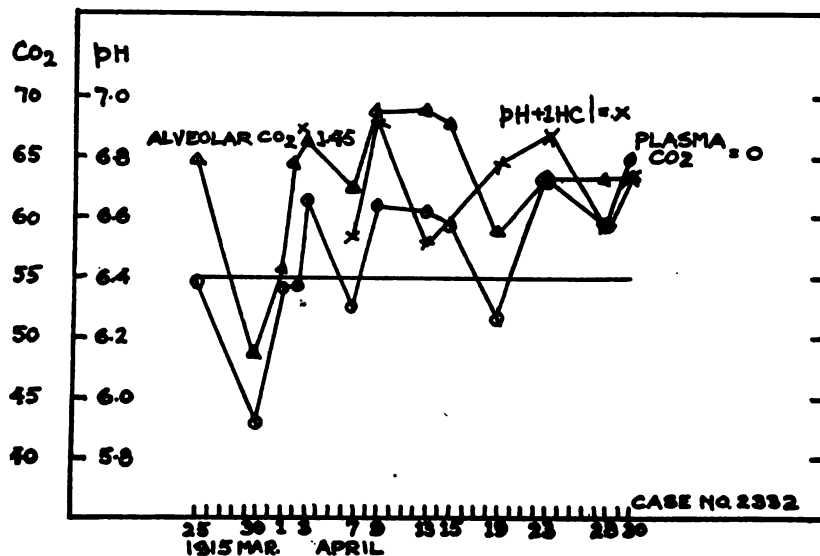
The curves are peculiar in that they are high during a period of relatively high fat feeding, and show an unstable and low level during a carbohydrate tolerance test, due perhaps to the 4 days' fast which preceded this test.

(From April 7-May 24, 1915.)

Group III.

14. Case 2332.

Date.	Total calories.	Protein.	Fat.	Carbohydrate.	Alcohol.	Alveolar CO ₂ .		CO ₂ bound by 100 cc. plasma at 20°.	Ratio plasma CO ₂ mm. alv. CO ₂	Plasma + 1 vol. 4/50 HCl.
		gm.	gm.	gm.	cc.	mm.	mm. X 1.45	cc.		pH
1915										
Mar.										
25	1,720	91.5	58	30	97.5	44.7	64.8	54.6	1.22	
26	1,725	85.5	61	"	"					
27	"	91.5	58.5	"	"					
28	682 Fast day.				"					
29	2,180	111.0	99.0	30	"					
30	2,215	111.5	102.5	"	"	33.3	48.3	42.7	1.28	
31	2,250	112.5	105.5	"	"					
Apr.										
1	2,215	112.0	102.5	"	"	38.5	55.8	54.4	1.41	
2	2,385	114.0	115.0	40	"	44.6	64.7	54.4	1.22	
3	"	"	"	"	"	45.9	66.6	61.5	1.34	
4	700 Fast day.				100.0					
5	2,370	116.0	111.0	40	"					
6	2,410	114.0	116.0	"	"					
7	2,890	115.0	167.0	"	"	42.9	62.2	52.4	1.22	6.46
8	2,660	"	"	"	67.5					
9	1,920	88.0	150.0	"		47.6	69.0	61.0	1.28	6.92
10	1,975	86.0	156.5	"						
11	157 Fast day.				22.5					
12	1,975	87.0	156.5	40						
13	2,025	90.5	156.0	50		47.6	69.0	60.6	1.27	6.50
14	2,060	90.0	160.0	"						
15	2,085	91.5	173.5	"		46.8	67.7	59.5	1.27	6.59
16	2,145	90.5	169.0	"						
17	2,095	89.0	164.0	"						
18	Fast day.									
19	2,035	91.5	156.5	50		40.1	58.2	51.4	1.28	6.78
20	1,975	89.0	155.5	40						
21	2,010	79.0	174.5	"						
22	1,955	89.0	153.5	"						
23	1,945	87.5	"	"		43.5	63.1	63.1	1.45	6.84
24	1,975	90.0	155.5	"						
25	Fast day.									
26	1,970	90.0	161.0	25						
27	2,000	"	165.0	"						
28	2,165	93.5	180.5	"		43.6	63.2	59.0	1.35	6.58
29	2,110	93.0	175.0	"						
30	2,140	92.5	178.5	"		43.7	63.4	64.9	1.49	6.74



14. Case 2332, age 42. Diabetic 8 years, of mild type, complicated by furunculosis and carbuncles.

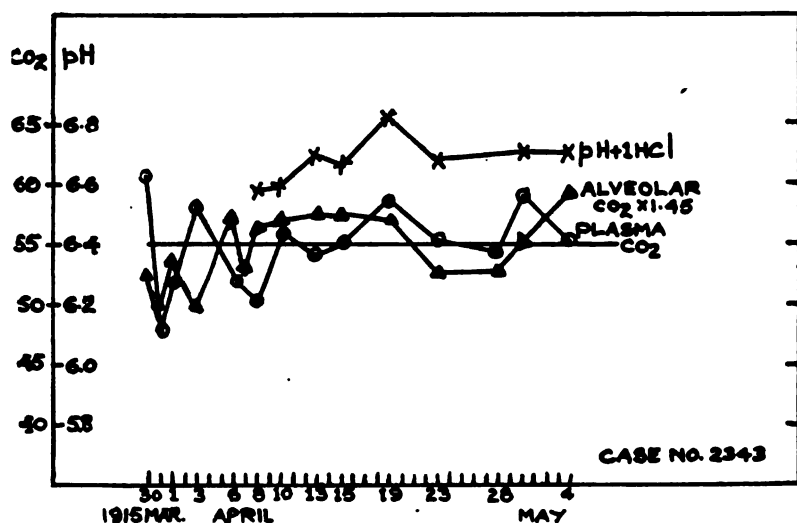
During the period over which the chart is drawn, the patient suffered a rather intense gastrointestinal upset of unknown origin. This is reflected in the instability of the curves. Following the recovery from this attack, the curves continue on a high level up to the time of discharge from the hospital.

(From March 25–April 30, 1915.)

Group I.

15. Case 2343.

Date.	Total calories.	Protein.	Fat.	Carbohydrate.	Alcohol.	Alveolar CO ₂ .		CO ₂ bound by 100 cc. plasma at 20°.	Ratio plasma CO ₂ mm. alv. CO ₂	Plasma + 1 vol. n/50 HCl.
1915		gm.	gm.	gm.	cc.	mm.	mm. × 1.45	cc.		pH
Mar.										
30	1,940	98.5	83.5	15.0	100	36.2	52.2	60.5	1.67	
31	1,940	80.5	91.0	"	100	33.9	49.2	47.8	1.41	
Apr.										
1	1,965	100.0	84.0	20.0	100	37.1	53.8	51.6	1.39	
2	1,965	82.0	91.0	"	100					
3	1,970	89.5	88.0	"	100	34.4	49.9	58.2	1.69	
4	1,960	97.0	84.5	"	100					
5	2,235	108.5	109.0	19.0	100					
6	2,245	120.0	104.5	20.0	100	39.3	57.0	52.0	1.32	
7	2,240	102.0	112.0	"	100	36.4	52.8	51.4	1.41	
8	2,065	102.0	112.0	"	75	38.8	56.2	49.3	1.27	6.57
9	1,490	97.5	104.0	30.0						
10	1,510	98.5	105.5	"		39.3	57.0	56.0	1.42	6.58
11	210 Fast day.				30					
12	1,725	97.5	129.5	30.0						
13	1,955	90.0	157.5	"		39.8	57.7	54.0	1.36	6.69
14	1,970	92.0	158.5	"						
15	2,205	92.0	183.5	"		39.5	57.3	55.0	1.39	6.66
16	2,090	97.0	179.5	"						
17	2,085	89.5	182.5	"						
18	Fast day.									
19	2,215	90.0	180.5	40.0		39.3	57.0	58.5	1.49	6.82
20	2,745	104.0	231.5	"						
21	2,730	95.0	234.0	"						
22	2,715	102.5	229.0	"						
23	2,710	101.5	229.0	"		36.9	52.5	55.2	1.50	6.68
24	2,715	102.5	230.0	"						
25	Fast day.									
26	2,665	97.0	231.0	30.0						
27	2,655	97.0	230.0	"						
28	2,775	90.0	245.5	"		36.4	52.8	54.4	1.49	
29	2,755	96.5	240.5	"						
30	2,760	92.0	243.0	"		38.0	55.1	59.0	1.55	6.70
31	2,735	94.0	239.0	"						
May										
1	Fast day.									
2	2,760	93.5	242.0	30.0						
3	2,780	97.0	243.0	"						
4	2,930	101.0	257.5	"		40.8	59.2	55.4	1.36	6.70
5	3,090	104.0	273.5	"						
6	3,090	104.0	273.5	"						
7	2,580	100.5	220.0	"						



15. Case 2343, male, age 44. Diabetic 9 years, mild in type, without evidence of acidosis before the institution of active treatment.

The curves show an unstable and rather low level, following a 2 months' period of very low diet, and a gradual rising and uniformity of the curves when the patient was placed on an adequate diet.

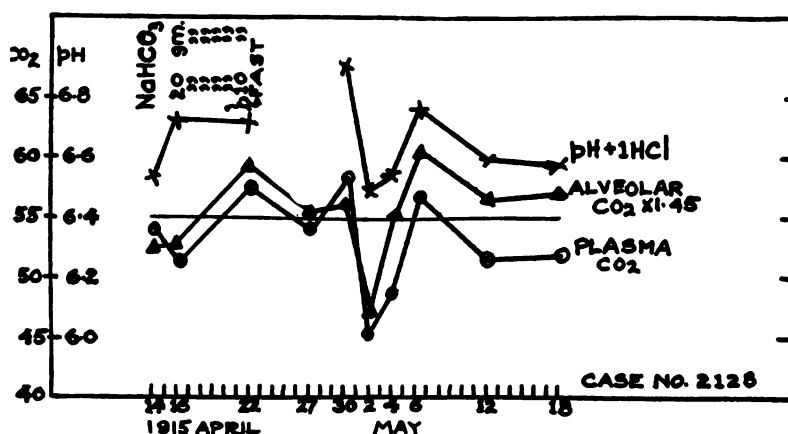
(From March 30-May 4, 1915.)

Group I.

16. Case 2128.

Date.	Total calories.	Protein.	Fat.	Carbohydrate.	NaHCO ₃ .	Alveolar CO ₂ .		CO ₂ bound by 100 cc. plasma at 20°.	Ratio plasma CO ₂ mm. alv. CO ₂	Plasma + 1 vol. HCl. N/50
1915		gm.	gm.	gm.	gm.	mm.	mm. × 1.45	cc.		pH
Apr.										
15	2,040	142	145	26.5	20	35.9	52.1	54.0	1.50	6.53
16	2,830	202.5	204	26.0	"	36.4	52.8	51.4	1.41	6.72
17	2,780	155.0	223	18.0	"					
18	1,845	129.0	133	19.0	"					
19	1,370	47.0	104.5	50.5	"					
20	Fast day.				"					
21	" "				10					
22	2,005	8.0	201.5	24.0		40.8	59.2	57.5	1.41	6.72
23	2,225	32.5	186.0	89.5						
24	2,430	40.5	202.0	95.0						
25	1,000	39.0	58.0	74.0						
26	Green veg. only containing			75.0						
27	"					38.3	55.5	54.0	1.41	
28	1,855	2.6	155.5	75.0						
29	"	"	"	"						
30	930	0	100.0	0		38.7	56.1	58.8	1.52	6.92
May										
1	1,440	29.5	141.5	1.0						
2	1,450	30.5	142.0	"		31.3	45.4	45.1	1.44	6.49
3	1,075	"	102.0	"						
4	1,040	26.0	100.0	"		38.0	55.1	48.6	1.28	6.55
5	815	24.0	80.0	"						
6	1,095	37.0	78.0	54.0		42.0	60.9	57.0	1.36	6.78
7	Fast day.									
8	1,095	32.5	67.0	83.0						
9	1,075	33.0	66.5	67.5						
10	1,060	29.0	66.0	81.0						
11*	175 Fast day.									
12	870	26.5	44.0	86.5		38.8	56.3	51.8	1.35	6.60
13	930	32.5	40.0	104.0						
14	875	29.5	40.0	93.0						
15	1,180	45.0	45.5	140.0						
16	465	0	50.0	0						
17	1,130	43.5	46.5	124.5						
18	950	42.0	29.0	123.5		39.3	57.0	51.9	1.32	6.57

* Alcohol, 25 cc.



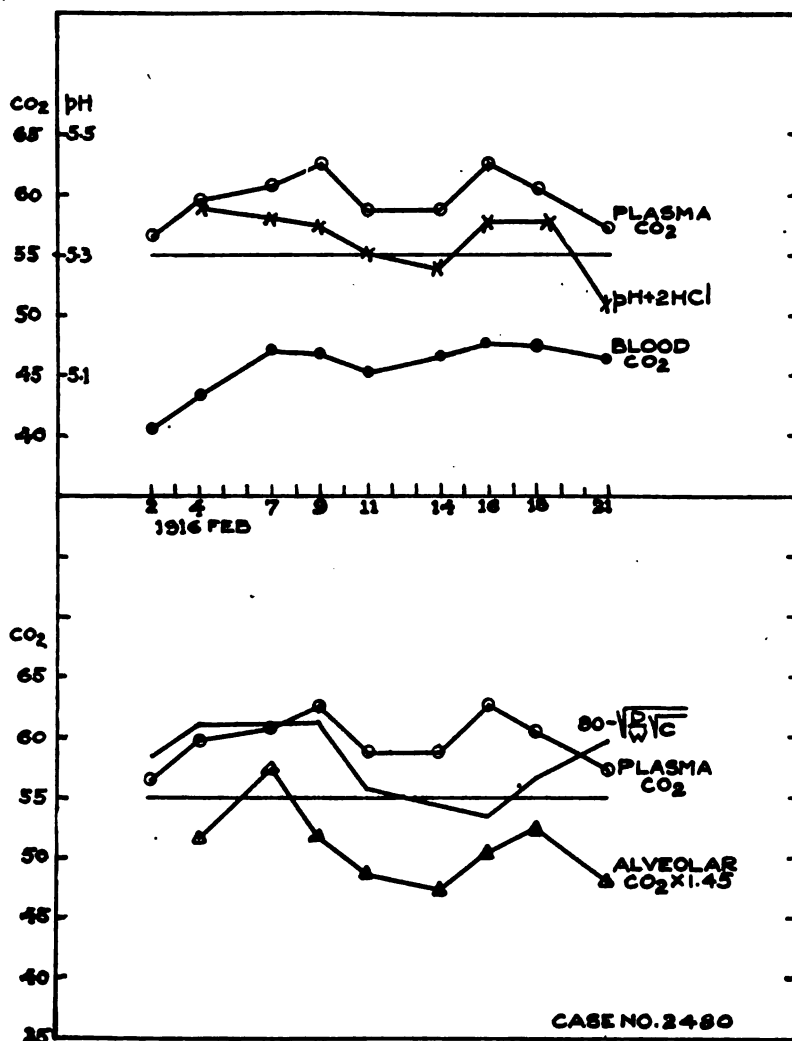
16. Case 2128, female, age 18. Diabetic 3 years, of severe type in inability to metabolize carbohydrate or fat, though maintaining a fair state of nutrition and strength.

(From April 15–May 18, 1915.)

Group III–IV.

17. Case 2480.

Date.	Diet.				Body weight. kg.	Alveolar CO ₂ .		Blood.				Urine.					$\sqrt{\frac{W}{D}}$	$80 - \sqrt{\frac{W}{D}}$
	Total calories.	Protein. gm.	Fat. gm.	Carbohydrate. gm.		mm.	mm. X 1.45	CO ₂ bound by 100 cc. plas- ma at 20°. cc.	Ratio plasma CO ₂ mm. alv. CO ₂	CO ₂ bound by 100 cc. whole blood at 37°. cc.	Plasma + 2 vol. HCl. N/50	Vol. per 24 hrs. cc.	Urea per liter. cc. N/10	NH ₃ per liter. cc. N/10	Acid per liter. cc. N/10	$\sqrt{\frac{W}{D}}$		
1916											pH							
Feb.	1,400	75	120	0	40.8			56.3		40.5		1,945	2,975	300	125	21.8	58.2	
2	"	"	"	2.5														
3	"	"	"	"														
4	"	"	"	"	40.5	35.5	51.3	59.6	1.68	43.1	5.38	2,065	3,435	251	120	19.0	61.0	
5	"	"	"	"														
6	Fast day.																	
7	1,300	65	110	0	39.1	39.4	57.2	60.6	1.54	47.0	5.36							
8	"	"	"	"														
9	"	"	"	"	40.8	39.2	51.8	62.4	1.59	46.8	5.35	2,195	3,000	275	120	18.8	61.2	
10	"	"	"	"														
11	"	"	"	"	41.0	33.5	48.6	58.8	1.76	45.0	5.31	1,250	4,620	540	180	24.2	55.8	
12	"	"	"	"														
13	Fast day.																	
14	1,300	65	110	0	40.8	32.7	47.4	58.8	1.80	46.6	5.28	920	4,890	540	184	20.9	59.1	
15	"	"	"	"														
16	"	"	"	"	41.4	34.6	50.2	62.7	1.81	47.7	5.36	1,625	3,685	460	132	26.7	53.3	
17	"	"	"	"														
18	"	"	"	"	41.8	36.0	52.2	60.3	1.68	47.4	5.36	1,095	5,010	586	196	23.5	56.5	
19	"	"	"	"														
20	Fast day.																	
21	1,100	65	90	0	41.4	33.4	48.0	57.1	1.71	46.3	5.22	1,400	3,375	433	140	20.4	59.6	



17. Case 2480, female, age 28. Diabetic 5 years, formerly of a mild type, but latterly developing into an obstinately progressive form, with emaciation and inability to tolerate calories enough to maintain existence in an aglycosuric state. A low grade acidosis has been present for 15 months of observation.

During the period shown the patient was temporarily free from acidosis as the result of unusual response to treatment. The acid excretion corresponds to the plasma bicarbonate, but the alveolar CO₂ is consistently too low.

(From February 2-21, 1916.)

Group III.

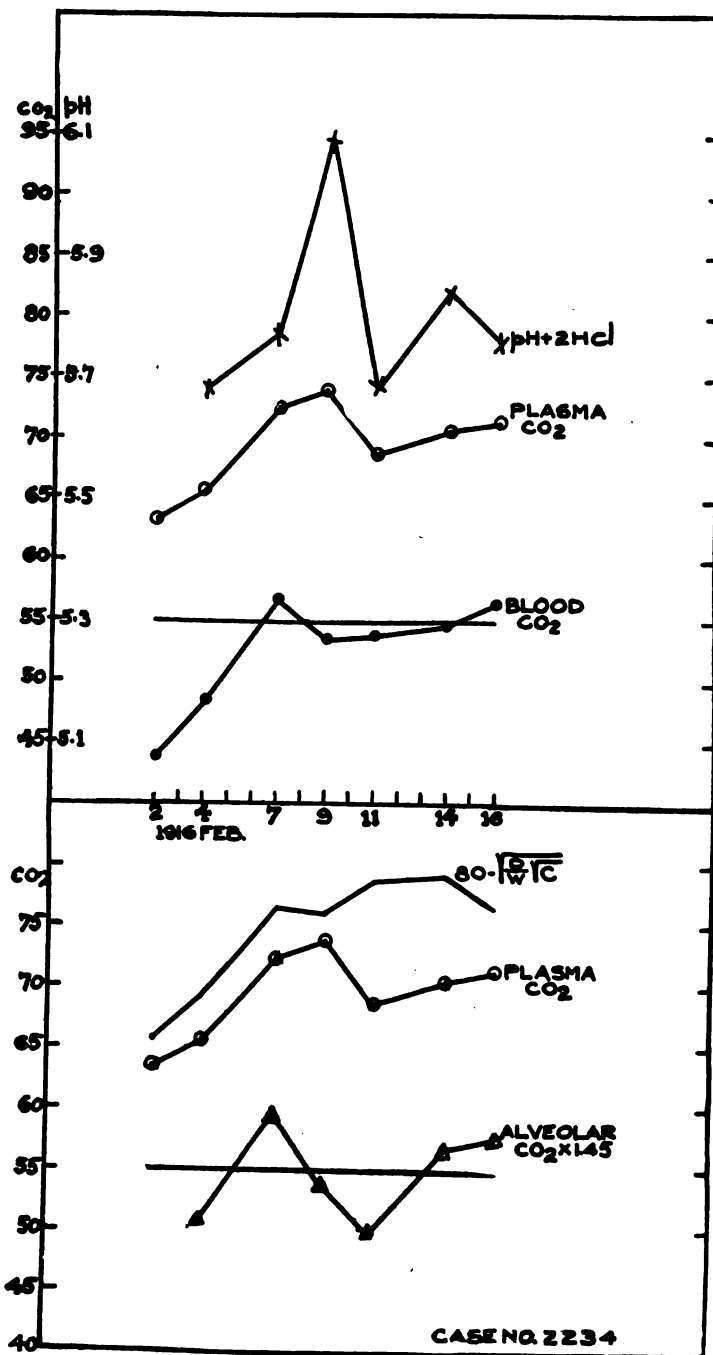
18. Case 2234.

Date.	Diet.				Body weight. kg.	Alveolar CO ₂ .		Blood.				Urine.					
	Total calories.	Protein.	Fat.	Carbohydrate.		mm.	mm. X 1.45	CO ₂ bound by 100 cc. plas- ma at 20°	Ratio plasma CO ₂ mm. alv. CO ₂	CO ₂ bound by 100 cc. whole blood at 37°	pH	Vol. per 24 hrs.	Urea per liter.	NH ₄ per liter.	Acid per liter.	$\sqrt{\frac{W}{D} \times C}$	$80 - \sqrt{\frac{W}{D} \times C}$
1916																	
Feb.																	
2	Green veg. only containing			20	48.0			63.1		43.7		740	3,380	270	300	14.2	65.8
3	" "			30													
4	" "			40	48.2	34.8	50.4	65.5	1.88	48.2	5.68	1,050	3,080	128	184	10.8	69.2
5	" "			50													
6	" "			60													
7	" "			70	48.0	40.9	59.3	72.3	1.77	56.3	5.77	2,275	1,575	22	34	3.4	76.6
8	" "			80													
9	" "			90	48.2	37.0	53.6	73.9	2.0	53.2	6.09	1,975	1,460	22	34	4.0	76.0
10	" "			100													
11	" "			110	48.6	34.2	49.6	68.8	2.01	53.9	5.68	1,890	1,525	23	26	1.8	78.2
12	" "			120													
13	" "			130													
14	" "			140	48.6	39.2	56.8	70.4	2.80	54.8	5.84	2,145	1,460	18	22	1.0	79.0
15	" "			150													
16	" "			115	49.2	39.6	57.4	71.1	2.80	56.1	5.76	1,635	2,030	20	32	3.0	77.0

18. Case 2234, male age 54. Diabetic 4 years, of the arteriosclerotic underweight type, albuminuric and cylindric, but with a normal urea index.

The chart shows the curve of typical mild diabetes without acidosis, on the diet of a carbohydrate tolerance test.
(From February 2-16, 1916.)

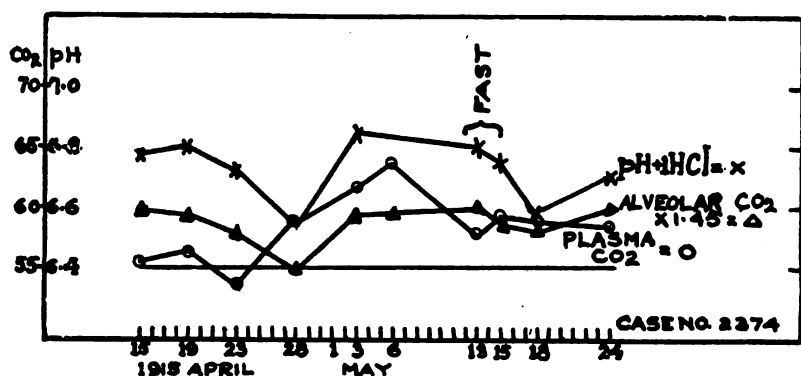
Group I.



19. Case 2374.

Date.	Total calories.	Protein.	Fat.	Carbohydrate.	Alveolar CO ₂		CO ₂ bound by 100 cc. plasma at 20°.	Ratio plasma CO ₂ min. alv. CO ₂	Plasma + 1 vol. N/50 HCl.
	gm.	gm.	gm.		mm.	mm. × 1.45	cc.		pH
1915 Apr. 15	Green veg. only containing			10	41.0	59.5	55.4	1.35	6.77
16	"	"	"	"					
17	"	"	"	"					
18	Fast day.								
19	700	25	57.5	15	40.8	59.2	56.4	1.38	6.80
20	1,190	44.5	101.7	"					
21	"	"	"	"					
22	1,380	63.5	114.0	"					
23	"	"	"	"	39.8	57.8	33.4	1.34	6.72
24	1,325	65.0	107.5	"					
25	Fast day.								
26	1,550	65.5	131.5	15					
27	1,605	64.0	138.0	"					
28	1,530	66.0	130.0	"	37.7	54.7	59.0	1.57	6.53
29	"	"	"	"					
30	1,595	"	136.0	"					
May 1	1,540	73.5	126.5	"					
2	Fast day.								
3	1,625	75.0	133.0	20	40.8	59.2	61.8	1.51	6.85
4	1,665	"	137.0	"					
5	1,705	67.5	145.5	"					
6	2,090	76.0	182.0	"	41.0	59.5	63.6	1.55	
7	1,975	72.5	175.0	13					
8	1,820	68.5	165.5	0					
9	Fast day.								
10	1,910	66.5	169.5	15					
11	503*	20.5	44.0	2					
12	Fast day.								
13	"	"	"	"	41.4	60.0	57.7	1.39	6.80
14	"	"	"	"					
15	675*	32.5	54.0	10	40.4	58.6	59.0	1.46	6.75
16	1,040	45.0	98.0	"					
17	1,250	63.5	102.5	"					
18	"	"	"	"	40.0	58.0	58.3	1.46	6.57
19	"	"	"	"					
20	1,260	72.5	99.5	"					
21	1,575	75.0	132.0	"					
22	1,805	83.5	153.5	"					
23	Fast day.								
24	1,570	57.0	140.0	10	40.4	60.0	58.5	1.41	6.71

* Partial fast.



19. Case 2374, age 61. Diabetic 11 years, of a mild type, apparently arrested until the year before admission, when, following a physical shock, the patient showed signs of marked acidosis.

The curves illustrate the absence of acidosis in a mild diabetic responding satisfactorily to treatment.

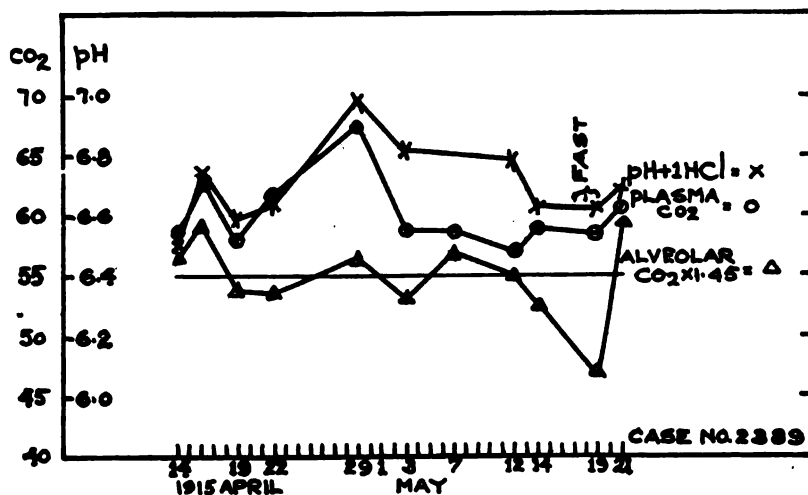
(From April 15-May 24, 1915.)

Group I.

20. Case 2389.

Date.	Total calories.	Protein.	Fat.	Carbohydrate.	Alveolar CO ₂ .		CO ₂ bound by 100 cc. plasma at 20°.	Ratio plasma CO ₂ mm. alv. CO ₂	Plasma + 1 vol. N/50 HCl.
1915		gm.	gm.	gm.	mm.	mm. X 1.45	cc.		pH
Apr. 14	1,805	46.5	165	20	38.8	56.2	58.5	1.51	6.50
15	1,860	50.0	"	30					
16	1,815	56.5	"	20	40.7	59.0	62.6	1.54	6.75
17	1,795	"	163.5	"					
18	Fast day.								
19	1,910	46.0	176.5	20	37.2	53.9	57.5	1.55	6.58
20	"	"	"	"					
21	"	"	"	"	37.1	53.8	61.6	1.66	6.65
23	"	"	"	"					
24	"	"	"	"					
25	Fast day.								
26	1,910	46.0	176.5	20					
27	"	"	"	"					
28	2,040	60.0	185.0	"					
29	2,025	67.0	180.5	"	39.0	56.6	67.2	1.72	6.99
30	"	"	"	"					
May 1	1,910	66.0	167.5	"					
2	Fast day.								
3	1,995	70.0	170.0	30	36.5	53.0	58.7	1.61	6.81
4	"	"	"	"					
5	1,990	61.0	173.5	"					
6	2,020	60.0	177.5	"					
7	2,025	81.0	168.5	"	39.3	57.0	58.8	1.50	
8	2,020	70.5	173.0	"					
9	Fast day.								
10	2,020	70.5	173.0	30					
11	2,045	80.0	171.0	"					
12	2,040	62.5	178.5	"	37.9	55.0	57.0	1.50	6.79
13	1,920	65.5	169.0	20					
14	1,930	69.5	172.5	"	36.1	52.4	59.0	1.63	6.62
15	"	"	"	"					
16	400*	20.0	34.0						
17	Fast day.								
18	"	"							
19	1,450*	34.0	136.0	11	32.5	46.4	58.2	1.82	6.62
20	1,805	45.0	168.0	15					
21	"	"	"	"	41.0	59.5	60.6	1.48	6.69

* Partial fast.



20. Case 2389, male, age 35. Diabetic $4\frac{1}{2}$ years, of moderate severity as shown by low tolerance for carbohydrate.

The curves show the absence of acidosis in a case which responds satisfactorily to treatment. The alveolar CO₂ following fasting on May 18–19, shows on the 19th a false drop, due not to fall in blood bicarbonate, but to the nervous effect of fasting.

(From April 14–May 21, 1915.)

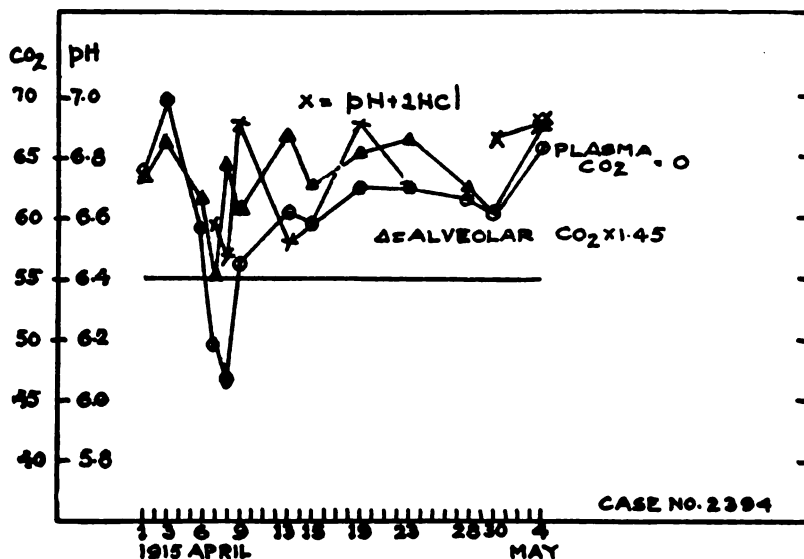
Group I.

21. Case 2394.

Date.	Total calories.	Protein.	Fat.	Carbohydrate.	Alveolar CO ₂ .		CO ₂ bound by 100 cc. plasma at 20°.	Ratio plasma CO ₂ mm. alv. CO ₂	Plasma vol. + 1 m/50 HCl.
1915		gm.	gm.	gm.	mm.	mm. X 1.45	cc.		pH
Apr. 1	1,175	34.5	50.0	150.0	43.6	63.2	63.2	1.45	
2	"	"	"	"					
3	1,300	40.5	45.0	175.0	45.9	66.7	69.8	1.52	
4	"	"	"	"					
5	Fast day.								
6	785	29.0	61.5	25.0	42.1	61.1	59.1	1.40	
7	1,290	50.0	106.0	"	37.7	54.7	49.4	1.31	6.59
8	1,425	70.0	111.5	"	44.6	64.7	46.3	1.04	6.47
9	1,780	69.0	150.0	"	41.7	60.5	56.4	1.35	6.92
10	"	"	"	"					
11	Fast day.								
12	2,110	76.0	171.5	50.0					
13	2,105	68.5	174.0	"	46.1	66.9	60.6	1.31	6.52
14	2,100	74.5	171.0	"					
15	2,545	71.0	220.0	"	43.2	62.6	59.5	1.38	6.60
16	2,585	76.5	221.0	"					
17	2,575	69.5	224.0	"					
18	Fast day.								
19	2,615	84.5	223.0	50	45.1	65.4	62.6	1.39	6.90
20	2,620	77.0	226.0	"					
21	2,640	80.0	228.5	"					
22	2,660	86.0	226.0	"					
23	2,710	95.5	217.5	"	45.7	66.3	62.1	1.36	6.71
24	2,715	85.0	232.5	"					
25	Fast day.								
26	2,590	84.0	219.5	50					
27	2,525	77.5	215.0	"					
28*	2,205	74.0	184.0	21	42.9	62.2	61.6	1.44	
29	Fast day.								
30	1,735**	60.5	161.0	22	41.5	60.2	60.3	1.45	6.86
May 1	2,385	80.5	210.5	25					
2	Fast day.								
3	2,380	80.0	210.0	25					
4	2,370	79.0	209.0	"	46.6	67.6	65.9	1.41	6.91

* Alcohol, 15 cc.

** Partial fast.

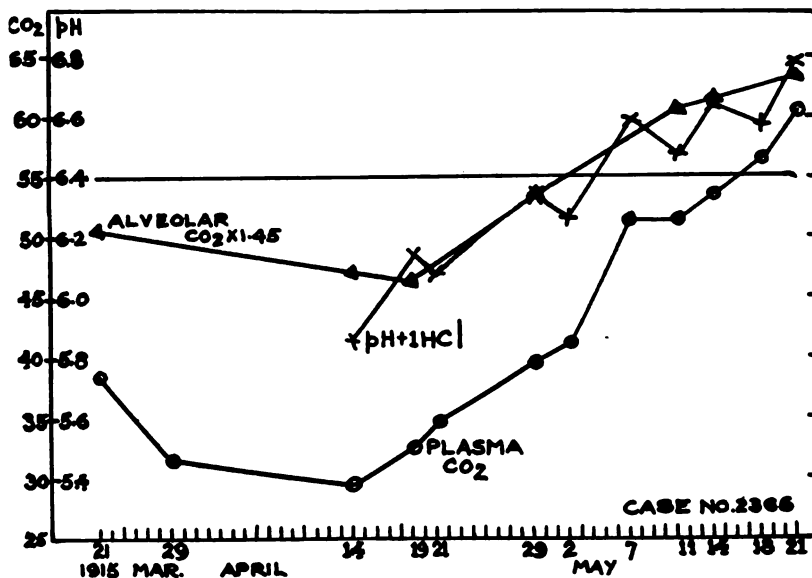


21. Case 2394, male age 30. Diabetic $1\frac{1}{2}$ years, of mild type without any evidence of acidosis.

The chart shows a fall in the curves to below normal during the period of adjustment following a high carbohydrate diet. Following this period the curves remained normal throughout.

(From April 1–May 4, 1915.)

Group I.



22. Case 2366, male, age 26. Lobar pneumonia, empyema, acute nephritis. Acute nephritis was discovered February 12, 1915, during convalescence from a severe attack of lobar pneumonia followed by empyema. Typical course of severe acute nephritis with evidence of marked change in renal function followed recovery.

The chart shows the curves in a patient with the acidosis of acute nephritis, with return to normal as renal function improved. These curves are of especial interest because they show an effect of disease on respiration exactly opposite to that often encountered in severe diabetes. The alveolar CO₂ was consistently much too high to indicate the real extent to which the alkaline reserve was lowered. (March 21-May 21, 1915.)

Nephritic.

METHODS FOR THE DETERMINATION OF PNEUMOCOCCUS TYPES.*

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It has been shown by Cole¹ and his associates that 75 to 80 per cent of cases of lobar pneumonia in adults are caused by three fixed types of highly parasitic pneumococci which can be differentiated readily by immunological methods, and that the remaining 20 to 25 per cent of cases are caused by a heterogeneous group of serologically independent varieties of pneumococci. These types have been classified by Dochez and Gillespie² primarily by means of animal protection experiments into Groups I, II, III (*Pneumococcus mucosus*), and IV (heterogeneous group). It has been shown that classification by agglutination corresponds to the classification by animal protection. Avery³ has further shown that there is a considerable group of pneumococci which are related to Group II organisms in that mice are protected against infection with such atypical Group II pneumococci by Type II antipneumococcus immune serum and in that such pneumococci are agglutinable by Type II immune serum, though less rapidly and completely than Type II pneumococci. Immune sera prepared by the immunization of animals against such atypical Group II pneumococci, however, fail to agglutinate Type II organisms or to protect mice against infection with Type II strains. These atypical Group II pneumococci have been classified as Subgroups IIa, IIb, and IIx (heterogeneous). No such atypical strains have been encountered in Groups I or III. Statistical studies have shown that the mortality rate in pneumonia varies with the type of pneumococcus causing the disease, the percentage of deaths in each group being fairly constant from year to year. In a series of 400 cases⁴ not receiving specific serum treatment the mortality rate was as follows: Group I, 25.2 per cent; Group II, 28 per cent; Group III, 56 per cent; Group IV, 14 per cent. These facts have be-

* This work was done under the tenure of a William O. Moseley Travelling Fellowship from Harvard University.

¹ Cole, R., *Arch. Int. Med.*, 1914, xiv, 56; *N. Y. Med. J.*, 1915, ci, 1, 58; *Tr. Cong. Am. Phys. and Surg.*, 1916, x, 138.

² Dochez, A. R., and Gillespie, L. J., *J. Am. Med. Assn.*, 1913, lxi, 727.

³ Avery, O. T., *J. Exp. Med.*, 1915, xxii, 804.

⁴ Moore, H. F., and Chesney, A. M., *Arch. Int. Med.*, 1917, xix, 611.

come a valuable guide in prognosis and have provided a basis for rational specific serum therapy in lobar pneumonia which at present has been successfully developed for cases of pneumonia caused by Type I pneumococci.

The determination of pneumococcus types in cases of lobar pneumonia is rapidly coming into extended use because of its value as a prerequisite for specific serum treatment and in the field of prognosis. It is essential that a reliable and standard technique for the determination of types should be used and that the method should be as rapid as possible in order that serum treatment, when indicated, may be instituted at the earliest possible moment.

The method in common use consists in the intraperitoneal injection into a mouse of a specimen of the patient's sputum. By this means a rapid growth of the pneumococcus is obtained while other secondary organisms are usually inhibited. After a suitable interval, varying in the individual case from 5 to 24 hours as determined by preliminary peritoneal puncture with a capillary pipette, the mouse is killed and the peritoneal exudate is washed out with 4 to 5 cc. of normal salt solution. The leukocytes and fibrin are removed from the peritoneal washings by centrifugalization at low speed; the supernatant bacterial suspension is decanted into a second centrifuge tube and whirled at high speed until the bacteria are thrown down. This supernatant fluid is discarded, and the bacterial sediment resuspended in normal salt solution. The type of pneumococcus present is then determined by macroscopic agglutination tests with undiluted antipneumococcus immune serum. At the time of mouse autopsy cultures of the peritoneal exudate and heart's blood are made in broth and on blood agar plates for subsequent confirmation of the determination of type made on the peritoneal washings.

Certain factors have interfered with the rapid determination of types by this method in an appreciable number of cases. The most frequent difficulty encountered has been the growth of secondary organisms in the peritoneal exudate together with the pneumococcus, notably *Bacillus influenzae* and less frequently *Micrococcus catarrhalis*, staphylococci, streptococci, and *Bacillus typhi murium*. When this occurs agglutination of the pneumococci present is either inhibited or markedly delayed, or spontaneous agglutination occasionally occurs in all tubes. A delay of 18 to 24 hours results until ag-

glutination tests can be made on pure cultures of the pneumococcus obtained from cultures made of the heart's blood at the time of mouse autopsy. When *Bacillus influenzae* or *Bacillus typhi murium* are present in the peritoneal exudate they are usually present also in the heart's blood cultures and further delay takes place through the necessity of fishing and culturing pneumococcus colonies from the blood agar plate.

A further difficulty which has been encountered recently with the agglutination technique commonly used has been the occurrence of a small number of strains of pneumococci properly belonging in Group IV which agglutinate in all three types of antipneumococcus serum. The character of the agglutination is less rapid and less complete than that which occurs with the fixed types of pneumococci, resembling the agglutination of Subgroup II pneumococci by Type II serum. This phenomenon of cross agglutination is one that might be expected from our knowledge of other bacterial groups and it in no way invalidates the classification of the parasitic pneumococci into sharply defined immunological groups. It rather indicates that with the use of undiluted serum of high agglutinin titer a zone of non-specific reaction is encountered. While only a relatively small number of pneumococcus strains exhibit the phenomenon of cross agglutination, it has been shown by the more delicate precipitin test that a limited zone of non-specific reaction exists for strains of pneumococci of all four groups. It is necessary to give but one protocol of a considerable series to demonstrate this (Table I).

TABLE I.

Determination of the Zone of Non-Specific Precipitin Reaction, Using Pneumococcus Type II Antigen and Antipneumococcus Immune Sera I, II, and III.

Type II antigen 0.5 cc.	1:100	1:500	1:1,000	1:5,000	1:10,000	1:50,000	1:100,000	1:300,000	1:500,000
Serum I (1:10) 0.5 cc.....	++	++	+	±	—	—	—	—	—
" II (1:10) 0.5 "	+++	+++	+++	+++	+++	+++	±	±	—
" III (1:10) 0.5 "	++	++	±	+	±	—	—	—	—
Normal horse serum (1:10) 0.5 cc....	—	—	—	—	—	—	—	—	—

The reactions were read after 2 hours at 37°C. and over night on ice. Pneumococcus II antigen was prepared by drying *in vacuo* the washed pneumococci from an 18 hour 1,000 cc. bouillon culture. The dried bacterial bodies were taken up in salt solution (10 mg. per cubic centimeter) and the suspension was repeatedly frozen and thawed until a faintly opalescent fluid free from bacterial bodies was obtained. Dilutions of the antigen were made in 0.85 per cent salt solution.

From Table I it is evident that a zone of non-specific precipitation occurs with Pneumococcus II antigen in dilutions not greater than 1:10,000 and that when higher dilutions are used the reaction becomes specific. Similar results have been obtained with antigen prepared from strains of Pneumococcus Type I and Pneumococcus Type III. Group IV pneumococcus antigens show a precipitin reaction only within the limits of the non-specific zone.

To obviate these difficulties certain improvements in the technique for the determination of pneumococcus types have been developed and will be presented below. The preliminary steps are the same as in the commonly used method and will be given in detail for the sake of completeness.

Inoculation of Mice with Sputum.

Collection of Sputum.—Care should be exercised in the collection of sputum to obtain a specimen from the deeper air passages as free as possible from saliva. This can be done in practically all cases, even the most difficult, with a little persistence. The physician or nurse should personally superintend the collection of sputum inducing the patient to cough until a suitable specimen is raised, care being taken not to allow the patient to swallow the lung sputum. The sputum is collected in a sterile Esmarch dish or other suitable container and should be sent at once to the laboratory for mouse injection. When delay is unavoidable the specimen should be kept on ice during the interval.

Microscopic Examination of the Sputum.—Direct films are made from the sputum and stained by Gram's method, with 10 per cent aqueous safranin as a counterstain, by Ziehl-Neelson's stain, and

by Hiss's capsule stain. This serves to give an idea of the nature of the organisms present and an indication of the source of the sputum. Suitable lung specimens are relatively free, in most instances, from contaminating mouth organisms. It is frequently possible to identify Type III (*Pneumococcus mucosus*) organisms when they are present, as they possess a very large distinct capsule staining by both Gram's and Hiss's methods.

Mouse Inoculation.—A small portion of the sputum, about the size of a bean, is selected and washed through three or four changes of sterile salt solution in sterile Esmarch or Petri dishes to remove surface contaminations. The washed sputum is then transferred to a sterile mortar, ground up, and emulsified with about 1 cc. of sterile bouillon or salt solution, added drop by drop, until a homogeneous emulsion is obtained that will readily pass through the needle of a small syringe. 0.5 to 1 cc. of this emulsion is inoculated intraperitoneally into a white mouse with a sterile syringe. The pneumococcus grows rapidly in the mouse peritoneum while the majority of saprophytic mouth organisms rapidly die off with the exceptions noted above, *Bacillus influenzae*, and occasionally *Micrococcus catarrhalis*, staphylococci, and streptococci. Pneumococcal invasion of the blood stream also occurs early. *Bacillus influenzae* like wise invades the blood stream if present; other organisms as a rule do not.

The time elapsing before there is a sufficient growth of the pneumococcus in the mouse peritoneum for the satisfactory determination of type varies with the individual case, depending upon the abundance of pneumococci in the specimen of sputum and the virulence and invasive power of the strain present. It may be from 5 to 24 hours, averaging 6 to 8 hours with the parasitic fixed Types I, II, and III. As soon as the injected mouse appears sick a drop of peritoneal exudate is removed by means of peritoneal puncture with a sterile capillary pipette, spread on a slide, stained by Gram's method, and examined microscopically to determine whether there is an abundant growth of the pneumococcus present. If there is an abundant growth of the pneumococcus present in pure culture the mouse is killed and the determination of type proceeded with. If the growth is only moderate or if other organisms are present in any quantity, further time must be allowed until subsequent examination of the

peritoneal exudate shows an abundant growth of the pneumococcus. It should be emphasized that undue haste in killing the mouse is time lost in the end.

Mouse Autopsy.—As soon as the mouse is killed or dies, the peritoneal cavity is opened with sterile precautions and cultures are made of the exudate in plain broth and on one-half of a blood agar plate. Films are made and stained for microscopic examination by Gram's stain and Hiss's capsule stain. The peritoneal exudate is then washed out by means of a sterile glass pipette with 4 to 5 cc. of sterile salt solution, the washings being placed in a centrifuge tube. Cultures are then made from the heart's blood in plain broth and on the other half of the blood agar plate.

Determination of Type.

Agglutination Method.—When the pneumococcus is present in pure culture in the peritoneal exudate the determination of type may be satisfactorily made by macroscopic agglutination tests as follows. The peritoneal washings are centrifugalized at low speed for a few minutes until the cells and fibrin contained in the exudate are thrown down. The supernatant bacterial suspension is decanted into a second centrifuge tube and centrifugalized at high speed until the organisms are thrown down. The supernatant fluid is discarded and the bacterial sediment taken up in sufficient sterile salt solution to make a moderately heavy suspension. The concentration of bacteria should be similar to that of a good 18 hour broth culture of the pneumococcus. This suspension is used directly for macroscopic agglutination tests, being mixed with immune serum in small test-tubes in equal quantities of 0.5 cc. each.

To obviate the difficulty that occasionally arises from the occurrence of Group IV strains that show cross agglutination in all three types of immune serum, the optimum dilutions of serum and the optimum incubation time that will surely identify all type strains and fail to give any cross agglutination reactions have been determined on a large series of strains.⁵ The results are shown in Table II.

⁵ This work applies only to the antipneumococcus immune serum prepared at the Hospital of The Rockefeller Institute for Medical Research.

TABLE II.
Determination of Pneumococcus Types by Agglutination.

Pneumococcus suspension 0.5 cc.	Serum I (1:20) 0.5 cc.	Serum II (undiluted) 0.5 cc.	Serum II (1:20) 0.5 cc.	Serum III (1:5) 0.5 cc.
Type I.....	++	—	—	—
“ II.....	—	++	++	—
Subgroups II a, b, x.....	—	+	—	—
Type III.....	—	—	—	++
Group IV.....	—	—	—	—

Incubation for 1 hour at 37°C.

From Table II it will be seen that a 1:20 dilution of Type I serum, making with the addition of an equal amount of pneumococcus suspension a final dilution of 1:40, a 1:20 dilution of Type II serum making a final dilution of 1:40, and a 1:5 dilution of Type III serum making a final dilution of 1:10, serve to agglutinate Types I, II, and III pneumococci respectively and fail to show any cross agglutination reactions with strains belonging in Group IV. It will further be seen that with 0.5 cc. of undiluted Type II serum, as well as with the 1:20 dilution, pneumococci belonging to the various Subgroups II may be identified and rapidly differentiated from Type II pneumococci in that they show partial to complete agglutination in undiluted Type II serum, but not in the 1:20 dilution at the end of 1 hour's incubation at 37°C.

For the determination of types of pneumococci in the peritoneal washings such serum dilutions give the most satisfactory and clear-cut results. Five small test-tubes are set up as follows: Tube 1, 0.5 cc. of Serum I (1:20) + 0.5 cc. of bacterial suspension; Tube 2, 0.5 cc. of Serum II (undiluted) + 0.5 cc. of bacterial suspension; Tube 3, 0.5 cc. of Serum II (1:20) + 0.5 cc. of bacterial suspension; Tube 4, 0.5 cc. of Serum III (1:5) + 0.5 cc. of bacterial suspension; Tube 5, 0.1 cc. of sterile ox bile + 0.3 cc. of bacterial suspension to determine the bile-solubility of the strain for differentiation from the streptococcus. The tubes are incubated in the water bath for 1 hour at 37°C. Agglutination of Types I, II, and III pneumococci in such serum dilutions is practically always immediate in the homologous serum and no agglutination occurs in the heterologous sera.

Rapid clumping of the organisms is seen to take place and may be brought out clearly by gentle agitation of the tubes. For the identification of Subgroup II pneumococci incubation is necessary, such strains showing partial to complete agglutination in undiluted Type II serum at the end of 1 hour's incubation. If no agglutination occurs and the organism is bile-soluble, it is classified as a Group IV pneumococcus.

Precipitin Method.—It has been stated above that the determination of pneumococcus types by macroscopic agglutination tests with the peritoneal washings is interfered with when other organisms are present, with a resultant delay of 18 hours or more before the type of pneumococcus present can be established. To obviate this difficulty the following method has been devised. Dochez and Avery⁶ have shown that the pneumococcus produces in broth cultures during the period of active growth a soluble substance which gives a specific precipitin reaction with the homologous antipneumococcus immune serum. It seemed probable that this soluble substance or precipitinogen would be present in the peritoneal exudate of the mouse in sufficient quantity to give a specific precipitin reaction with the homologous serum and such has proved to be the case. The method to be described is dependent upon this phenomenon.

The peritoneal exudate is washed out with 4 to 5 cc. of sterile salt solution by means of a sterile glass pipette and placed in a centrifuge tube. The peritoneal washings containing cells, fibrin, and bacteria are immediately centrifuged at high speed until the supernatant fluid is water clear. The supernatant fluid is then pipetted off, with care not to disturb the sediment, which is discarded, and is mixed in quantities of 0.5 cc. each with an equal amount of the antipneumococcus immune serum in a series of small test-tubes as follows: Tube 1, 0.5 cc. of Serum I (1:10) + 0.5 cc. of supernatant peritoneal washings; Tube 2, 0.5 cc. of Serum II (undiluted) + 0.5 cc. of supernatant peritoneal washings; Tube 3, 0.5 cc. of Serum II (1:10) + 0.5 cc. of supernatant peritoneal washings; Tube 4, 0.5 cc. of Serum III (1:5) + 0.5 cc. of supernatant peritoneal washings. An immediate specific precipitin reaction occurs in the tube contain-

⁶ Dochez, A. R., and Avery, O. T., *Proc. Soc. Exp. Biol. and Med.*, 1916-17, xiv, 75.

ing the homologous immune serum, the other tubes remaining clear (Table III). No incubation is necessary. Two tubes of Type II serum are used for the purpose of distinguishing between Type II pneumococci and members of the Subgroups II, the former giving a precipitin reaction in both tubes, the latter only in the undiluted Type II serum. A negative reaction in all tubes indicates a pneumococcus belonging in Group IV.

The method has been tested with a large number of strains and has been consistently positive and specific with pneumococci of Types I, II, and III and consistently negative with pneumococci of Group IV. The presence of other organisms together with the pneumococcus in the peritoneal exudate does not interfere with the reaction and other organisms than the pneumococcus produce no substance that might give a false positive reaction.

TABLE III.

Determination of Pneumococcus Types by the Precipitin Method.

Supernatant peritoneal washings 0.5 cc.	Serum I (1:10) 0.5 cc.	Serum II (undiluted) 0.5 cc.	Serum II (1:10) 0.5 cc.	Serum III (1:5) 0.5 cc.
Type I.....	++	—	—	—
“ II.....	—	++	++	—
Subgroups II a, b, x.....	—	+	—	—
Type III.....	—	—	—	++
Group IV.....	—	—	—	—

The results with Subgroup II pneumococci have not been so satisfactory. Reference to Table III will show that pneumococci belonging to these groups give a precipitin reaction with undiluted Type II serum but not with the 1:10 dilution, thereby being distinguished from Type II pneumococci. A number of Subgroup II organisms, however, have been encountered in which the peritoneal washings have failed to give a precipitin reaction with undiluted Type II serum. In the identification of the fixed parasitic types of pneumococci this occasional difficulty is of little practical importance from the point of view of treatment as there is at present no specific therapy for cases of pneumonia caused by pneumococci of the Subgroups II. For purposes of classification and statistics these organisms can be

readily identified subsequently when the organism has been obtained in pure culture.

In order to determine the shortest time after mouse inoculation in which a clear-cut positive precipitin reaction may be obtained and whether it occurs as soon as a satisfactory agglutination test can be made, a series of mice have been inoculated intraperitoneally with measured amounts of pure pneumococcus cultures and the mice have been killed at varying intervals. Simultaneous determinations of type have been made by both the agglutination and precipitin methods on each peritoneal washing. The results are shown in Table IV.

TABLE IV.

Comparison of Agglutination and Precipitin Methods.

Mouse.	Time killed after inoculation with 0.01 cc. of culture.	Series A, Type I.		Series B, Type II.		Series C, Type III.	
		Agglutination.	Precipitin.	Agglutination.	Precipitin.	Agglutination.	Precipitin.
	<i>hrs.</i>						
1	4	—	—	—	—	—	—
2	5	—	—	—	—	—	+
3	6	—	—	—	+	—	++
4	7	—	+	—	++	—	++
5	8	++	++	++	++	++	++

From these experiments it is evident that the agglutination method possesses no advantage in point of time over the precipitin method. The presence of the soluble precipitinogen in the peritoneal exudate in sufficient quantity to give a clear-cut precipitin reaction coincides in the case of Type I pneumococci with the earliest time when satisfactory agglutination tests can be made. With Type II pneumococci it occurs earlier and with Type III still earlier. This phenomenon exhibits an interesting parallelism with the capsular formation and virulence of the three types of pneumococci.

The precipitin method possesses the following distinct advantages. It is available as soon as satisfactory agglutination tests can be made; incubation of the tubes is unnecessary; it is not interfered with by the presence of other organisms in the exudate; it is specific and shows no cross immunity reactions; it is applicable to mice which

through unavoidable circumstances have been dead for some time before the determination of type can be made and in which autolysis of the pneumococci or postmortem invasion of the peritoneal cavity by other organisms has made the agglutination method impracticable. For these reasons it is recommended as the method of choice in all cases.

Identification of Type III Pneumococci by Morphological and Cultural Characteristics.—If Type III antipneumococcus immune serum is not available for diagnostic purposes Type III pneumococci may be identified in most instances by cultural and morphological characteristics. *Pneumococcus mucosus* is usually somewhat larger, rounder, and less lanceolate than other types of pneumococci. It possesses a large distinct capsule which stains readily with Hiss's capsule stain and usually retains the pink counterstain with Gram's method. The peritoneal exudate produced on mouse inoculation is usually quite mucoid and colonies on solid media are moist, mucoid, and spreading. It is always bile-soluble. These characteristics usually serve to differentiate Type III pneumococci from other types. Occasional strains of pneumococci which agglutinate in Type III serum, however, are encountered which do not show well developed mucoid characteristics and cannot be distinguished culturally from other types. Furthermore, Type II strains are occasionally met with that exhibit fairly well developed mucoid characteristics. For these reasons the identification of Type III pneumococci by morphological and cultural characteristics is not always absolute, and the diagnosis should be established by immunological methods when Type III serum is available.⁷

Confirmation of Type.

The determination of type on the peritoneal washings should be confirmed by macroscopic agglutination tests with a pure bouillon culture of the pneumococcus obtained from culture of the heart's blood at the time of mouse autopsy. The technique is the same as that employed in the agglutination tests on the bacterial suspension obtained from the peritoneal washings and should include a test for bile-solubility.

⁷ Wadsworth, A. B., and Kirkbride, M. B., *J. Exp. Med.*, 1917, xxv, 629.

Determination of Types of Pneumococci in Blood Cultures, Spinal Fluids, Empyema Fluids, and by Lung Puncture.

Blood Culture.—The usual technique in routine blood cultures is carried out. From a positive bouillon blood culture 10 cc. are removed by pipette and centrifugalized at low speed to remove the blood cells. The supernatant fluid is pipetted off and the bacteria are thrown down by centrifugalization at high speed, the supernatant fluid is discarded, and the bacterial sediment is suspended in sterile salt solution. The pneumococcus type is then determined by macroscopic agglutination tests following the same technique described above.

Spinal Fluid and Empyema Fluid.—Cultures are made by the methods ordinarily employed in culturing fluids and the type of pneumococcus is determined when the culture has grown out by the use of the same technique as that applied to blood cultures. If desired, in addition to culturing spinal fluids, a portion of the fluid may be centrifugalized at high speed to throw down the pneumococci present, and the sediment, taken up in 1 cc. of sterile salt solution, inoculated intraperitoneally into a mouse.

Lung Puncture.—This procedure should be resorted to only when it is impossible to obtain a suitable specimen of sputum or a positive blood culture. Cultures are made in bouillon of the lung puncture material and the determination of type is made by the same technique as that employed in the case of blood cultures.

Determination of Pneumococcus Types by Direct Sputum Culture.

It was thought that the determination of types of pneumococci by the precipitin method might be possible by direct culture of the washed sputum in bouillon without resort to mouse passage. Tubes of bouillon were inoculated with specimens of sputum and incubated 6 to 8 hours at 37° C. The cultures were then centrifugalized at high speed until the supernatant bouillon was clear, and precipitin tests were made by mixing equal parts of the supernatant bouillon and immune serum. In a few instances sufficient precipitinogen had been produced by the growth of the pneumococcus in the culture to give a positive precipitin reaction with the homologous serum. This by

no means invariably occurred with all specimens of sputum, however, and seemed to depend largely upon the number of pneumococci present in the sputum and to some extent upon the type of pneumococcus. The number of experiments done was small and the results were not sufficiently satisfactory to make the method of practical use. It is possible that further experiments along these lines might develop a technique which would prove available for use as a routine method.

SUMMARY.

The determination of pneumococcus types in lobar pneumonia is of value in the field of prognosis and as a prerequisite for specific serum therapy. The method for the determination of types should be as rapid as possible and a standard technique should be employed. The most satisfactory method is by the intraperitoneal inoculation of a mouse with the patient's sputum, by which means a rapid and abundant growth of the pneumococcus is obtained and secondary organisms are rapidly eliminated in most instances. The diagnosis of type is made directly on the peritoneal exudate. Certain factors in the method commonly used have interfered with the rapid determination of types in an appreciable number of cases, notably the growth of other organisms in the peritoneal exudate together with the pneumococcus, and some confusion has arisen because occasional strains of pneumococci have been encountered that show cross agglutination reactions when undiluted immune serum is used. Such reactions have been shown to be due to a limited zone of non-specific immunity and they in no way invalidate the classification of the pneumococci into sharply defined immunological groups. The optimum dilutions of serum have been determined that will agglutinate all type strains of pneumococci and fail to cause any cross agglutination reactions when mixed with equal amounts of pneumococcus cultures and incubated for 1 hour at 37°C. They are a 1:20 dilution of Serum I, a 1:20 dilution of Serum II, and a 1:5 dilution of Serum III. For the diagnosis of Subgroup II pneumococci undiluted Type II serum is required.

To obviate the other difficulties of the method commonly used a new method for the determination of types has been devised. It

depends upon the fact that there is produced by the growth of the pneumococcus a soluble substance which is present in the peritoneal exudate of the mouse in sufficient quantity to give a specific precipitin reaction with the homologous immune serum. The precipitin method can be used in all instances in which the determination of types by the agglutination method is possible, and it possesses certain distinct advantages which make it available when the agglutination method is impracticable. It is of particular value as a time-saving device in those instances where the presence of other organisms together with the pneumococcus in the peritoneal exudate causes a delay of 18 hours or more before the type of pneumococcus can be definitely established. It is therefore recommended as the method of choice in all cases. If desired, both the agglutination and precipitin methods may be applied to the same specimen of peritoneal washings.

SOME ANALYSES OF VEGETABLES SHOWING THE EFFECT OF THE METHOD OF COOKING.*

BY ANGELIA M. COURTNEY, HELEN L. FALES, AND FREDERIC H. BARTLETT, M. D.

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The addition of green vegetables to the dietary of young infants is becoming a common practice. It is generally believed that the value of this is due to the effect on the mineral metabolism. Infants who are fed for too long a time on an exclusive milk diet show anemia, late closure of the fontanel, delay in walking and general lack of activity. The secondary anemia is easily explained by the small amount of iron in cow's milk; after its dilution in milk formulas the amount of iron becomes almost negligible. Instead of making use of drugs to supply the needed iron, green vegetables have been fed to many children in the wards and the outpatient department of the Babies' Hospital. This plan has been followed with children as young as 6 or 7 months. It has been interesting to observe that infants thus fed show an earlier closure of the fontanel and generally greater activity than those without such additions to their diet.

How this beneficial effect is produced is a nutritional problem which is not within the scope of this paper. It has been variously explained. The mere addition of an increased quantity of salts may be advantageous. The particular combinations of the bases with the inorganic and organic acids present may provide the salts in an especially suitable form for use by the organism. A third view is that the value of the vegetables lies in a biologic or so-called vitamin effect. Since the general opinion is that the mineral content is the important factor, we have studied the subject from this point of view. We hoped to shed a little light on the question by determining the mineral metabolism of infants suffering with rickets and those showing delayed develop-

* Read at the meeting of the American Pediatric Society, White Sulphur Springs, W. Va., May 28, 1917.

ment, comparing their salt retention with and without vegetable additions to the diet.

The question immediately arose as to what was the actual mineral content of the vegetables as ordinarily prepared and administered. Abundant figures are available which show the total mineral content of the edible part of various vegetables. It is obvious, however, that the water used in cooking extracts more or less of the constituents and it is not a common practice to give this with the vegetable. Hence it is evident that the reported analyses are of no value in estimating the mineral content of vegetable additions to the diet of children. We therefore undertook a series of analyses of cooked vegetables, considering separately the solid portion ordinarily given as food and the water used in cooking.

At first the vegetables were cooked in the manner usual when preparing them as food for children; that is, by a very thorough boiling. After cooking, the vegetables were drained in a colander and the resulting solids and water were analyzed separately. The figures here reported are computed on the basis of 100 gm. of the edible portion of the uncooked vegetable (approximately a quarter of a pound).

TABLE 1.

Content in Grams of Solids of Vegetables Prepared by Boiling.

Vegetable	Minutes Boiled	Solids	Ash	CaO	MgO	P ₂ O ₅	Cl	K ₂ O	Na ₂ O	H ₂ SO ₄	Fe ₂ O ₃	Total N	N, as Protein
Spinach.....	90	8.30	1.172	0.305	0.035	0.123	0.036	0.238	0.068	0.034	0.0090	0.497	3.10
New Zealand spinach	30	4.26	0.535	0.145	0.021	0.052	0.000	0.157	0.040	0.016	0.0154	0.236	1.48
Young carrots.....	30	6.31	0.408	0.039	0.014	0.043	0.023	0.181	0.038	0.022	0.0070	0.108	0.67
Onions.....	45	6.82	0.398	0.020	0.013	0.067	0.008	0.186	0.010	0.056	0.0026	0.189	1.18
String beans.....	150	5.31	0.371	0.070	0.030	0.063	0.045	0.123	0.011	0.190	1.19
Asparagus.....	30	4.59	0.370	0.038	0.021	0.101	0.024	0.174	0.001	0.025	Trace	0.283	1.77
Potatoes.....	30	20.51

Table 1 shows the analyses of the solid portion of various vegetables boiled as described above.

Spinach shows the highest total salts, containing from two to three times as much ash as any of the other vegetables studied. This preponderance is due largely to calcium and phosphorus. The iron is

highest in the New Zealand spinach, the ordinary spinach and carrots being next. The iron in onions is very low, while asparagus has only a trace. This table plainly shows that spinach is the most efficacious in supplying mineral addition.

New Zealand spinach is a comparative newcomer in the market. It does not belong to the same family as the ordinary spinach. It possesses the advantage of being available for a continuous supply throughout the summer, when ordinary spinach is not easily available. Although there is considerable waste in the preparation of New Zealand spinach and it is rather a watery vegetable, it serves as a very satisfactory substitute for common spinach.

The analyses of the water drained from the cooked vegetables show very striking results. These are given in Table 2.

TABLE 2.

Percentage Lost in Water Under Ordinary Boiling Conditions.

Vegetable	Minutes Boiled	Solids	Ash	CaO	MgO	P ₂ O ₅	Cl	K ₂ O	Na ₂ O	H ₂ SO ₄	Fe ₂ O ₃	Total N
Spinach.....	90	32.2	45.2	Trace	61.5	48.2	71.1	64.8	61.1	57.2	28.2	23.1
New Zealand spinach.....	30	41.3	72.2	3.6	81.0	70.2	100.0	81.9	77.8	78.7	50.8	22.3
Young carrots.....	30	37.5	47.8	28.4	41.6	34.6	57.1	47.3	48.8	49.9	Trace	22.2
Onions.....	45	22.5	28.0	26.1	10.6	24.6	31.4	29.2	0	31.6	Trace	19.8
String beans.....	150	31.8	43.4	21.4	54.1	42.7	46.8	55.2	56.3	26.7
Asparagus.....	30	27.4	46.7	26.6	40.1	34.6	46.4	49.2	Trace	52.1	Trace	24.1
Potatoes.....	30	4.4

The excessive waste of salts if this water is discarded is very evident. It ranges from over a quarter of the total ash of onions to nearly three quarters of that of New Zealand spinach. In the others the loss is about half the total. Calcium is the only constituent which is not seriously affected. The examination of potatoes showed so small a loss of total solids that the analyses were not carried through.

An attempt was next made to reduce the loss by boiling the vegetables for minimum time. A comparison of the effect of long and short boiling is given in Table 3.

TABLE 3.

Comparison of Percentage Lost in Water with Long and Short Boiling.

Vegetable	Minutes Boiled	Solids	Ash	CaO	MgO	FeO ₃	Cl	K ₂ O	NaO	H ₂ SO ₄	FeO ₂	Total N
Spinach.....	90	32.2	45.2	Trace	61.5	48.2	71.1	64.8	61.1	57.2	28.2	23.1
Spinach.....	10	26.7	42.2	Trace	55.8	43.3	66.9	58.1	55.7	39.3	23.4	19.2
Beans.....	150	31.8	43.4	21.4	54.1	42.7	46.8	55.2	56.3	26.7
Beans.....	60	28.2	39.4	12.2	43.3	40.9	25.4	53.9	44.1	22.2

This table shows that the saving of salts by shorter boiling was insignificant. The only exceptions to this were the calcium and chlorid in the beans and the sulphate in spinach. Apparently the salts are lost early in the process of boiling.

Some modification of the method of cooking seemed to be necessary. Steaming was therefore tried, because it affords a method in which the vegetables are held apart from the boiling water instead of soaking in it as in boiling. For this purpose a rice steamer was used. The vegetables are held in a tightly covered receptacle with a rather finely perforated bottom. This part fits closely on the top of the boiler which holds the water. Any type of steamer which holds the vegetables out of the water would undoubtedly serve as well.

Table 4 shows the results obtained by cooking the vegetables in this way.

It is obvious from an examination of Table 4 that steaming is by far the most economical method of cooking for preserving the salts. The remarkable saving of salts should be emphasized. In spinach the loss by steaming becomes about half what it was in boiling. In asparagus it is less than a third, and in carrots it is not even a fourth. The effect of steaming on the different constituents of the New Zealand spinach is more variable than in case of the other vegetables. Sodium is lost in a high degree even in steaming, but the saving in the other constituents, except calcium, is very striking. Onions, which lost the least in boiling, were the least affected by the changed method of cooking. It is a strange fact that there is a slightly greater loss of calcium by steaming than by boiling, but since the calcium loss is small in any case, this is of little importance.

TABLE 4.

Comparison of Percentage Lost in Water by Steaming and by Boiling.

Vegetable	Method of Cooking	Minutes Cooked	Solids	Ash	CaO	MgO	P ₂ O ₅	Cl	K ₂ O	NaO	H ₂ SO ₄	FeO ₂	Total N
Spinach.....	Boiling.....	10	26.7	42.2	Trace	55.8	43.3	66.9	58.1	55.7	39.3	23.4	19.2
Spinach.....	Steaming...	15	17.5	25.5	(a)	(a)	19.7	(a)	34.8	51.2	(a)	(a)	(a)
New Zealand spinach.....	Boiling.....	30	41.3	22.2	3.6	81.0	70.2	100.0	81.9	77.8	78.7	50.8	22.3
New Zealand spinach (b)...	Steaming...	30	5.2	15.3	7.9	22.3	29.8	7.3	19.4	50.5	21.3	0	0
Young carrots....	Boiling.....	30	37.5	47.8	28.4	41.6	34.6	57.1	47.3	48.8	49.9	Trace	22.2
Young carrots....	Steaming...	15	8.8	13.6	(a)	(a)	10.7	(a)	10.0	14.3	(a)	Trace	(a)
Onions.....	Boiling.....	45	22.5	28.0	26.1	10.6	24.6	31.4	29.2	0	31.6	Trace	19.8
Onions.....	Steaming...	30	20.4	25.5	27.9	7.9	21.6	28.2	26.6	0	23.2	Trace	18.7
Asparagus.....	Boiling.....	30	27.4	46.7	26.6	40.1	34.6	46.4	49.2	Trace	52.1	Trace	24.1
Asparagus.....	Steaming...	30	8.5	15.2	(a)	(a)	11.5	(a)	15.8	0	(a)	(a)	(a)
Potatoes.....	Boiling.....	30	4.4
Potatoes.....	Steaming...	30	1.6

(a) The amount of solids in the water from the steamed vegetables was too small to permit making all determinations.

(b) An analysis of the water from the steamed New Zealand spinach was not made. The percentages lost were estimated by assuming the total content of the water and the solid portion to be the same in the steamed as in the boiled.

For the benefit of those who may wish to feed the vegetables steamed, Table 5 is presented.

As in Table 1, the figures in Table 5 are computed on the basis of 100 gm. of the edible portion of the uncooked vegetable. When cooked, this amount yields approximately three tablespoonfuls of all but carrots, which furnish four.

A comparison of Tables 1 and 5 makes impressive the greater concentration in salts of vegetables prepared by steaming. For example, from the same amount of New Zealand spinach one can obtain approximately three times as much mineral matter by steaming as is available by boiling.

TABLE 5.

Content in Grams of Solids of Vegetables Prepared by Steaming.

Vegetable	Minutes Stm'd	Solids	Ash	CaO	MgO	P ₂ O ₅	Cl	K ₂ O	Na ₂ O	H ₂ SO ₄	FeO ₂	Total N	N, as Protein
Spinach (c)	15	7.12	1.047	0.100	0.089	0.079	0.032	0.341	0.047	0.052	0.041	0.421	2.63
New Zealand spinach	30	6.88	1.628	0.139	0.085	0.124	0.009	0.701	0.090	0.059	0.037	0.305	1.91
Young carrots	15	9.50	0.669	0.047	0.025	0.063	0.047	0.276	0.074	0.019	0.014	0.137	0.86
Onions	30	6.70	0.402	0.019	0.016	0.070	0.010	0.195	0.012	0.057	0.003	0.199	1.24
Asparagus	30	5.93	0.556	0.030	0.026	0.145	0.040	0.285	0.001	0.039	Trace	0.373	2.33
Potatoes	30	22.82

(c) The spinach given here was a different variety from that used for the boiling experiment. This kind had thick, curly, dark green leaves and its total mineral content was much lower.

A table follows (Table 6) in which the approximate content of one teaspoonful of spinach and of carrots, cooked by steaming, has been estimated from Table 5.

TABLE 6.

Approximate Content of One Tablespoonful of Steamed Vegetable.

Vegetable	Solids	Ash	CaO	MgO	P ₂ O ₅	Cl	K ₂ O	Na ₂ O	H ₂ SO ₄	FeO ₂	Total N	N, as Protein
Spinach	2.37	0.349	0.033	0.030	0.026	0.011	0.114	0.016	0.017	0.014	0.140	0.88
Young carrots	2.40	0.167	0.012	0.006	0.016	0.012	0.069	0.018	0.005	0.004	0.034	0.21

If a steamer is not available, the cooking may be done in a double boiler. The vegetable should be prepared as usual, drained after washing and placed with no additional water in the inner boiler. Spinach requires about thirty minutes to cook in this manner. Table 7 gives a comparison of the result of this method of cooking with that by steaming.

It will be seen that there is very little choice between the two methods as regards the saving of salts.

Early in the paper reference was made to the possible importance of the combinations of bases with inorganic and organic acids. A study of the tables shows a great preponderance of bases over inor-

TABLE 7.

Comparison of Spinach Steamed and That Cooked in Double Boiler.

	Method of Cooking	Solids	Ash	CaO	MgO	P ₂ O ₅	Cl	K ₂ O	Na ₂ O	H ₂ SO ₄	Fe ₂ O ₃	Total N	N. as Protein
Content of solid portion from 100 gm. of spinach	Steaming	7.12	1.047	0.100	0.089	0.079	0.032	0.341	0.047	0.052	0.041	0.421	2.63
	Double boiler	8.03	0.997	0.166	0.049	0.107	0.024	0.243	0.114	0.574	3.59
Percentage lost in water of cooking	Steaming	17.5	25.5	(a)	(a)	19.7	(a)	34.8	51.2	(a)	(a)	(a)
	Double boiler	15.5	25.6	4.5	33.3	25.6	(a)	34.7	30.3	(a)	30.8	(a)

(a) The amount of solids in the water was too small to permit making all determinations,

ganic acids. This must mean that a considerable proportion of the bases are held as salts of organic acids. It is generally believed that such compounds are more assimilable than inorganic salts. Hence there would appear to be some ground for the theory that the beneficial effect of vegetable feeding is at least partly due to the character of the combinations of bases and acids.

Some metabolism experiments on the effect of vegetable addition to the food on the mineral balance are under way. With one child somewhat rachitic and very backward in development, an improved balance was obtained after spinach had been added to a food otherwise considerably reduced. We hope to report these results, with those from other observations, later.

The methods used in obtaining the results reported in this paper are those previously used in work reported from these laboratories.¹ The iron was determined gravimetrically as iron phosphate.

CONCLUSIONS.

1. A large proportion of the mineral content of most vegetables is lost in the water used in cooking by boiling.

1. Holt, L. E., Courtney, A. M., and Fales, H.: Chemical Composition of Diarrheal as Compared with Normal Stools in Infants, *AM. JOUR. DIS. CHILD.*, 1915, 9, 213.

2. This loss is only slightly reduced by making the time of boiling a minimum.

3. A very great saving in mineral content may be effected by using the method of steaming.

4. Spinach is the best vegetable to provide a salt addition to the diet.

OBSERVATIONS ON TYPES OF RESPONSE IN TREATMENT OF SYPHILIS OF THE CENTRAL NERVOUS SYSTEM.*

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In a short discussion of treatment of syphilis of the central nervous system it is practically impossible to consider all phases of the subject. With the recent simultaneous development of new methods of diagnosis and new forms of treatment, our knowledge of the results of therapy must necessarily rest upon an experimental basis. The object of this communication is, therefore, to present detailed observations in the treatment of various forms of the disease and to point out certain conclusions from these observations.

It is well established that the central nervous system is involved in all stages of the disease. While it is thought by many syphilographers that the future course of the disease is largely determined by the invasion of the nervous tissues in the early secondary period, still the various clinical pictures depend upon numerous factors, such as time since infection, amount of treatment, tissues involved, and certain unknown influences in resistance and immunity at present but little understood. In the days when nosology dominated the study of the disease, various types, such as syphilis and parasyphilis, were described. This classification was useful, and even today when combined with the more modern methods of laboratory examination, is not without value in prognosis and to a certain extent as a guide in treatment.

For convenience in classifying, the various types may be grouped under three main headings:

1. *Vascular*.—The essential lesion is an endarteritis, and the nervous lesion is due to disturbed circulation.

* Read before College of Physicians, Philadelphia, February 7, 1917.

2. *Exudative*.—The most marked lesion is cellular thickening of the supporting membranes or perivascular spaces, with gumma formation and subsequent mechanical injury to cortex, tracts, and intradural portions of nerves.

3. *Parenchymatous*.—The striking picture is tract or cortical degeneration, but in which the essential lesion is probably a chronic meningitis and perivascularitis. At least this appears to be true of the various tabetic manifestations. In paresis a true inflammation of the cortex seems to exist. This peculiarity in the paretic process probably explains the intractability of this condition to treatment.

The pessimism of many of the older neurologists in regard to permanent cure of central nervous syphilis probably arose because of their failure to take into consideration all of these factors, indeed many of the differences have been only recently appreciated. On the other hand the statement that all forms of central nervous syphilis respond equally well to intensive general medication must be the result of insufficient observation of a sufficiently wide variety of cases. By the presentation of the details of the treatment of a number of patients, I hope to prove that treatment must be individualized, and that the response in the cerebrospinal fluid is one of the most important guides to both the kind, and sufficiency of treatment.

The order in which the records are presented is determined to a certain extent by the stage of the disease. This order, however, is modified by various factors, such as clinical forms and peculiarities in laboratory findings.

Case 1 (W. E.) is an illustration of severe involvement of the meninges in a very early stage. With the appearance of headaches, even before the cutaneous manifestations, and deafness two months after the appearance of the chancre, we are justified in assuming that the eighth nerve was implicated in a basal meningitis. The results of single early injections of salvarsan are well demonstrated for although the symptoms were temporarily relieved, the essential process was not eliminated as shown by the first lumbar puncture. The ease with which the abnormal elements in the fluid may be eliminated by systematic treatment is well shown, for following eight intravenous injections of salvarsan in three months' time the fluid became practically normal. There was a simultaneous disappearance of symptoms

Group I.—Early Meningitis.

TABLE I.

W. E. Male. Age 25. Oct. 18, 1911. Primary.
 Nov. 15. Severe headache, hyperesthesia of scalp.
 Nov. 28. Salvarsan, amount unknown.
 Dec. 26. Deafness, right ear; headache.
 Jan. 22, 1912. Salvarsan, amount unknown.
 April 11. Admitted. Headache and nervousness. Physical examination negative except exaggerated reflexes and hyperemia optic discs.

DATE	BLOOD	CEREBROSPINAL FLUID			TREATMENT	
	W. R.	Cells	Noguchi Glob.	Wassermann Reaction	Salvarsan	Mercury
1912						
Apr. 16	++	190	±	0.8 c.c. +++++		
May 6 to May 20					3 × 0.3 gm.	
May 22	++++	23	±	1.0 c.c. +++++		
May 29 to June 11					3 × 0.3 gm.	
June 19 to June 27					2 × 0.6 gm. neo.	
July 2	++	7	—	1.0 c.c. —		
July 11					0.6 gm. neo.	4 inj. Hg. Sal.
Sept. 6	++++	4	—	1.0 c.c. —		
Sept. 9 to Sept. 23					5 × 0.9 gm. neo.	25 rubs
Nov. 22	++	0	—	1.0 c.c. —		
Nov. 25 to Jan. 1 1913					6 × 0.9 gm. neo.	3 inj. Cal.
Apr. 8	++++	5	—	1.0 c.c. —		
Apr. 1 to May 27					8 × 0.5 gm.	2 inj. Hg. Sal.
Sept. 30	+	1	—	1.0 c.c. —		
Oct. 1 to Feb. 16					12 × 0.5 gm.	4 inj. Hg. Sal.
1914						
Feb. 16	—					
Apr. 27	—	1	—	1.0 c.c. —	0.5 gm.	
1915						
Feb. 3	—	6	—	2.0 c.c. —		
Feb. 15	—				0.4 gm.	

TABLE I—*Concluded.*

DATE	BLOOD	CEREBROSPINAL FLUID			TREATMENT	
	W. R.	Cells	Noguchi Glob.	Wassermann Reaction	Salvarsan	Mercury
1915						
Feb. 16	—					
Feb. 19	+					
Feb. 25	+					
Mar. 1 to May 15						30 rubs
May 15	—					
1917						
Jan. 15	—	6	—	2.0 c.c. — Gold Curve: 1111100000		

A year and half of continuous intensive treatment was required, however, before the serum Wassermann reaction remained negative.

The manner in which this patient responded to treatment is fairly typical of that usually seen in early meningitis. Six out of a group of eight such cases have shown a similar response to persistent intensive general therapy. Several of them likewise showed a tendency for the symptoms to recur when early treatment was applied in a desultory manner.

Case 2 (C. W.) is an example of a true neuro relapse. The patient did not have any symptoms of central nervous origin until an interval had elapsed after the first course of salvarsan. Then a clinical picture appeared which pointed to severe and extensive involvement of the basal meninges. The general nervous symptoms were as severe as often seen in the second stage of tuberculous meningitis, and the cerebrospinal fluid showed a pleocytosis more intense than is often present in this disease.

The clinical improvement and diminution in abnormal elements in the cerebrospinal fluid promptly followed the administration of repeated injections of 0.2 gm. of salvarsan. The diminution in pleocytosis was slower than in Case 1. Twice, March 8, and October 26, 1912, there was an increase in the cell count while the patient was

TABLE II.

C. W. Male. Age 34. May 2, 1911. Primary.
 May 27. Secondaries.
 June 9 to 24. 1.5 gm. salvarsan.
 July–August. 6 inunctions and 12 inj. Hg.
 Aug. 19. Stiffness of neck, headache, dizziness.
 Aug. 23. Deafness, facial paralysis.
 Sept. 23. Admitted. Headache, dizziness, mental dullness, exaggerated reflexes, deafness, facial paralysis.

DATE	BLOOD	CEREBROSPINAL FLUID			TREATMENT	
	W. R.	Cells	Neguchi Glob.	Wassermann Reaction	Salvarsan	Mercury
1911						
Sept. 23	++++	1094	++	0.2 c.c. ++++		
Sept. 27 to Oct. 12	++++	118	+	0.2 c.c. —	3 × 0.2 gm.	
Oct. 16	++++				7 × 0.2 gm.	
Oct. 19 to Nov. 29	+++	60	+	1.0 c.c. ++	2 × 0.2 gm.	
Dec. 5	++					
Dec. 7 to Dec. 14						
1912						
Jan. 8 to Jan. 15						2 inj. Hg. Sal.
Jan. 21	—	9				
Jan. 21 to Mar. 4						6 inj. Hg. Sal.
Mar. 8	+	46	+	1.0 c.c. +		
Mar. 12 to Apr. 16	—	11	=	1.0 c.c. —	6 × 0.3 gm.	
Apr. 18	—				3 × 0.3 gm.	
May 7 to May 28	—	8	—	1.0 c.c. —		
May 31						6 inj. Hg. Sal.
July 16 to Oct. 7						
Oct. 19	+				0.9 gm. neo.	
Oct. 26	++++	22	—	1.0 c.c. —	7 × 0.9 gm. neo.	
Oct. 26 to Dec. 14						7 inj. Cal.
1913						
Jan. 13 to Mar. 25	+	6	+	1.0 c.c. —	3 × 0.9 gm. neo.	
Apr. 12					6 × 0.5 gm.	
Mar. 29 to Apr. 19						6 inj. Hg. Sal.
Apr. 26 to June 21						
June 29 to Aug. 18						

TABLE II—*Concluded.*

DATE	BLOOD	CEREBROSPINAL FLUID			TREATMENT	
	W. R.	Cells	Noguchi Glob.	Wassermann Reaction	Salvarsan	Mercury
1913						
Aug. 30	—	3	±	1.0 c.c. —	16 × 0.5 gm.	
Sept. 13 to Feb. 21 1914	—	6	±	1.0 c.c. —		
Feb. 21	—				9 × 0.5 gm.	
Apr. 25 to Sept. 7 1915	—					
Jan. 21	—				Gold Curve: 0000000000 0.3 gm. Sal. provocative	
June 12	—		—	2.0 c.c. —		
July 3 1916	—	7	—	2.0 c.c. —		
Oct. 23	—					
Oct. 25	—					
Oct. 28	—					
Oct. 30	—					
Nov. 2	—					
Nov. 7	—					

receiving intramuscular injections of mercury. This fact is of especial interest in connection with the occurrence of his first attack during intensive mercurial treatment. Only by persistent application of salvarsan over a period of three years with occasional short courses of mercury, could the spinal fluid be brought to a permanent normal condition. The persistence of a normal cerebrospinal fluid and of a negative serum Wassermann reaction during two years without treatment, and the negative results of provocative treatment seem to indicate that the disease is completely eradicated. This case illustrates how important the repeated examinations of the cerebrospinal fluid may be, for the recurring pleocytosis under mercury served as an indicator of the necessity for salvarsan. The superiority of salvarsan over mercury is also elicited. This is doubtless the type of case which terminated fatally from meningitis in the presalvarsan era.

Case 3 (W. R.)* is an example of how apparently a lesion of the central nervous system may be a focus from which the serum Wassermann reaction may repeatedly relapse. Three times during a period of two and one-half years the reaction reappeared in the serum when treatment was discontinued. The patient was able to take mercury in large quantities, and each subsequent course of treatment was more intensive than the last. The only symptoms or signs of central nervous system involvement were very active reflexes. Still in October, 1914, a lumbar puncture revealed the presence of an active meningitis. The form of treatment was then changed to less intense general therapy with the addition of intraspinal injections of the patient's own serum obtained after intravenous salvarsan injections. Under this new mode of treatment, there was steady improvement in the condition of the cerebrospinal fluid until it became entirely normal. Two more combined treatments were given after which all drugs were discontinued. Contrary to the experience in the first two years the blood Wassermann reaction has not relapsed and, moreover, the fluid has remained normal.

This case seems to prove that in certain patients even in the secondary period intensive general treatment may not be sufficient to control an infection in the cerebrospinal axis, and that intraspinal therapy in addition may be necessary.

These three cases illustrate the variations in response in the secondary period. The first was easily influenced by salvarsan intravenously, and there was no tendency to a relapse when treatment was discontinued. The second was more resistant and relapsed when mercury was substituted for salvarsan. In the third the most intensive general application of salvarsan, mercury, and iodides was not sufficient to eliminate the meningeal lesion. Only after intraspinal injection of serum was the active syphilitic lesion eliminated.

In all the patients the only clinical residuum of their nervous lesion is the persistence of very active deep reflexes. It is conceivable that as a result of the former inflammatory process there may be a certain amount of scarring which has affected the upper motor neuron.

* The patient was under the care of Dr. A. R. Stevens, who kindly furnished the notes.

TABLE III.
W. R. Male, Age 34.

DATE	BLOOD		TREATMENT					
	W. R.		Salvarsan	Hg. Sal. Intramuscular	HgCl ₂ pills p. o.			
1912								
Jan. 29		Primary lesion.						
Mar. 29		Rash. Adenopathy, Mucous patches.						
30 to			3 inj.	13 inj.				
June 25		Total:	1.65 gm.	Total: 1 gm.	2.5 gm.			
July 29	—							
Sept. 19	++							
Oct. 5 to			7 inj.	18 inj.				
1913			Total:	Total: 3.3 gm.	9.4 gm.			
Mar. 29			4.2 gm.					
May 21	—							
July 16	+++							
23	++++±							
28 to				6 inj.				
Sept. 20	—			Total: 0.8 gm.	2.6 gm.			
20 to			7 inj.	8 inj.				
Nov. 28		Total:	4 gm.	Total: 1.3 gm.	2.4 gm.			
28 to			4 inj.	10 inj.				
1914		Total:	2.4 gm.	Total: 2 gm.	2.6 gm.			
Feb. 25								
25 to			3 inj.	8 inj.				
May 18		Total:	1.7 gm.	Total: 1.6 gm.	3.3 gm.			
July 7	—							
Aug. 10	+++++							
12 to					2.6 gm.			
Oct. 6								
		CEREBROSPINAL FLUID		TREATMENT				
		Cells	Glob.	W. R.	Gold	Sal. Intrav.	Serum Intrap.	HgCl ₂ pills
Oct. 9		65	+	0.4 c.c. ++++				
24						0.6 gm.	20 c.c. 40%	
Nov. 6						0.6 gm.	30 c.c. 45%	
Dec. 4		30	++	0.8 c.c. ++++		0.6 gm.	52 c.c. 50%	
1915								
Jan. 8						0.6 gm.		
29			+	1.0 c.c. ++++		0.6 gm.	32 c.c. 40%	14.6 gm.
Mar. 26 to						0.5 gm.		KI 133
May 1								gm.
June 4		20		0.8 c.c. ++++		0.6 gm.	40 c.c. 50%	
July 9						0.5 gm.	40 c.c. 50%	
30						0.6 gm.	40 c.c. 50%	
Aug. 13		5	±	1.0 c.c. —		0.6 gm.	40 c.c. 50%	

TABLE III—*Concluded.*

DATE	BLOOD	CEREBROSPINAL FLUID				TREATMENT		
	W. R.	Cells	Glob.	W. R.	Gold	Sal. Intrav.	Serum Intrasp.	HgCl ₂ pills
Oct. 1			±	2.0 c.c. ++++		0.5 gm.	40 c.c. 50%	5.6 gm.
29			—	2.0 c.c. ++++		0.6 gm.	36 c.c. 50%	
Nov. 19						0.45 gm.	40 c.c. 50%	
Dec. 18		3	+	1.5 c.c. ++++		0.35 gm.	36 c.c. 50%	
1916					00000			
Jan. 28			—	2.0 c.c. —	00000	0.4 gm.	40 c.c. 50%	
Feb. 5								
Mar. 17			—	2.0 c.c. —				
May 20	—	3	—	2.0 c.c. —	00000 00000	0.4 gm.	30 c.c. 40%	
Aug. 1	—	5	—	2.0 c.c. —				
Nov. 26	—	2	—	2.0 c.c. —	00000 00000			

Case 4 (M. P.) is an example of a patient in whom the first nervous symptoms appeared nearly four years after infection. For the first two years he was under the care of Professor Milian, of Paris. During this time he received fairly intensive antisyphilitic treatment. During the eighteen months before admission to the Rockefeller Hospital, his treatment was more desultory in character. When first seen he had evidently a fairly active meningitis and retrobulbar neuritis with much narrowing of the visual fields. Because of his previous intensive arsenic treatment, it was felt that the effect of mercury and iodides should be tested before resorting again to an arsenical. For this reason the patient received mercury and iodides for three months with marked improvement in the condition of the visual fields, but at the same time, the cerebrospinal fluid showed no tendency to become normal. Following a course of full doses of neosalvarsan, and a short course of mercury intramuscularly, the cerebrospinal fluid became normal, the visual fields remained normal, and the fundi showed no abnormality except slight narrowing of the arteries. The provoking of a strong Wassermann reaction from a negative phase during the course of neosalvarsan is noteworthy. Because of the persistence of the Wassermann reaction in the blood serum, treatment

Group II.—Later Forms of Central Nervous Syphilis of the Exudative Type.

TABLE IV.

M. P. Male. Age 26. Syphilitic meningitis. Retrobulbar neuritis and atrophy.

Jan. 5, 1909. Chancre, spirochete present.

Jan. 5 to Mar. Arsacetin 30 inj. hypo.

Mar., 1909, to Mar., 1910. Hg. intramusc. 100 inj.

Mar. to July. Pills 100.

July, 1910, to July, 1911. Pills and KI.

July, 1911. Salvarsan intramuscularly, suppuration.

Jan., 1912. Salvarsan intramuscularly.

July, 1912. Sodium cacodylate 18 inj. Fullness in head, flashes of light, pain in legs.

Oct. 20, 1912. Admitted. Retrobulbar neuritis and atrophy, narrowing of visual fields.

DATE	BLOOD	CEREBROSPINAL FLUID			TREATMENT		
	W. R.	Cells	Glob.	Wassermann Reaction	Salvarsan gm.	Hg.	
1912							
Oct. 25	—	40	+	1.0 c.c. —			
27						Hg. Sal.—14 inj.	
to 1913						Total 0.8 gm.	
Jan. 23						KI 4 gm. per day	
Jan. 23	—	59	=	0.6 c.c. +++++			Visual fields normal.
Jan. 28	—				Neo. 0.6		
Feb. 4	=				" 0.9		
11	=				" 0.9		
15	++++				" 0.9		
22	++++				" 0.9		
Mar. 1	++++				" 0.9		
8	++				" 0.9		
15	+++				" 0.9		
22					Sal. 0.5		
Apr. 4 to						Hg. Sal. 0.5 gm.	
June 16						Total	
21	+++	4	—	1.0 c.c. —			Fundi and visual fields normal.
28					Sal. 3 × 0.4 gm.		

TABLE IV—*Concluded.*

DATE	BLOOD	CEREBROSPINAL FLUID			TREATMENT		
	W. R.	Cells	Glob.	Wassermann Reaction	Salvarsan gm.	Hg.	
1912							
July 12	++						
Sept. 18		7	—	2.0 c.c. —	Sal. 0.5 gm.		
1914							
Jan. 6 to						Hg. Sal. Total:	
Feb. 9						0.5 gm. hypo.	
23	+				5 × 0.5 gm.		
May 25	—						
1915							
June 21	—	1	+	2.0 c.c. —			Fundi normal color, arteries narrow. Fields normal.

was continued for another ten months. The patient then disappeared from observation, but upon his return a year later it was found that his general condition was normal and that the only abnormality in the cerebrospinal fluid was a slight increase in globulin. This persistence of excess globulin has been seen in many cases in whom all other pathological elements of the cerebrospinal fluid were entirely lacking. This case is an illustration of the fact that it is safe to use large and repeated doses of salvarsan in patients with marked involvement of the optic nerve, and also that the exudative type frequently responds to persistent general medication.

Case 5 (D. Y.). This is an example of the marked exudative type of lesion occurring many years after infection. It is probable from the history of the case that the patient contracted a marital infection at least twenty years ago. Except for headaches she had never suffered symptoms referable to disease of the central nervous system until two months before admission. At the time of the first examination, both the clinical findings and the cerebrospinal fluid gave evidence of a marked increase in intracranial pressure. In this type of case we feel that it is wise to introduce treatment with mercury and

TABLE V.

D. Y. Female. Age 51. Syphilis of cerebral meninges. Infection (?)
 Oct. 10, 1915. Headache, weakness of left leg and left face, thickness of speech, improved. For past 2 weeks progressive stupor, headaches, weakness.
 Nov. 30, 1915. Admitted. Disorientation. Rigid neck, double choked disc, exaggerated reflexes, incontinence of urine, fever.

DATE	BLOOD	CEREBROSPINAL FLUID				TREATMENT		
	W. R.	Cells	Glob.	W. R.	Gold	Sal. gm.	Hg. gm.	KI gm.
1915								
Dec. 2	++++	161	++	0.6 c.c. ++++			11 rubs	
4-17						0.25		229
17	++++					0.3	3 rubs	
17-28								
30	++++	50	+	2.0 c.c. ++++				103
to								
Jan. 8						0.3		
15				Discharged much improved				
Feb. to							Rubs—14 per mo.	"some"
June							for 4 mos.	
June to							No treatment	
Dec. 17		Readmitted. Anemia. Gastric ulcer.						
					11221			
Dec. 19	++++	4	=	2.0 c.c.	00000			

large doses of iodides before resorting to salvarsan, in order to eliminate the possibility of a Herxheimer reaction. In one month's time there was remarkable improvement in the clinical signs, a diminution in the choking of the discs, and a very gratifying improvement in the condition of the cerebrospinal fluid. After six weeks of treatment the patient insisted upon going home and continued treatment there for four months. Treatment was then stopped and after two or three months, there were symptoms pointing to the presence of a gastric ulcer. She was readmitted in December, 1916, with headaches, severe anemia, vomiting, and hematemesis. It was thought that this might be due to a gastric crisis, but further investigation confirmed the diagnosis of gastric ulcer, and on Lenhartz' diet, and after two transfusions, the headaches disappeared and the patient's condition was

much improved. At the time of her second admission, the cerebrospinal fluid was normal except for a slight globulin excess and a very weak luetic type of gold curve.

The case is a striking example of satisfactory improvement in the cerebrospinal fluid and clinical symptoms which was effected largely by the use of mercury and iodides.

TABLE VI.

I. C. Female. Age 30. Cerebral syphilis 3 mos. Infection (11 yrs.?).
 Oct., 1916. Convulsions, unconsciousness followed by strabismus and diplopia.
 Dec. 26, 1916. Admitted. Unequal pupils. Ptosis of right lid. Strabismus. Right choked disc, left optic neuritis. Left facial paralysis.

DATE	BLOOD	CEREBROSPINAL FLUID				TREATMENT			
	W. R.	Cells	Glob.	Wassermann Reaction	Gold	Intrav. Sal.	In-trasp. serum	Hg. gm.	KI gm.
1916 Dec. 28 to Jan. 8	+++	10	++	1 c.c. ++++	55555 42100			HgCl ₂ 5 × 0.012 4 rubs	56 158
11	+++					0.1 gm.			
18	++++	2	+	2 c.c. +		0.1 gm.			
25	++++	4	+	2 c.c. -	11232 11000	0.25 gm.	15 c.c. 100%		

Case 6 (I. C.). While the exact time of infection can not be determined in this case, it was probably about eleven years before admission. The case was interesting because of the uncertainty in diagnosis. With an initial symptom of convulsions and unconsciousness, the diagnosis of paresis was highly suggestive. The physical examination, however, revealed a condition which was more easily explainable by a diffuse basilar process involving both optic nerves, the oculomotor nerves on both sides and the left facial nerve. The low cell count was unusual with extensive meningitis. The gold curve, however, was of a typical parietic type. As with the former patient, treatment was instituted by means of mercury and iodides. Because of salivation after a short course of mercury, small doses of salvarsan were

given. After three weeks of treatment there was a marked improvement in the cerebrospinal fluid and after four weeks the cell count was normal, the globulin less and the Wassermann reaction negative. However, because of the initial gold curve of the paretic type, it was felt that the most intensive treatment should be instituted at once, hence intraspinal injections of serum were given. The treatment was followed by a disappearance of the strabismus, marked improvement in the ophthalmoscopic picture, and general clinical betterment. Both the clinical improvement and the tendency of the cerebrospinal fluid to become normal, have been comparable in rate to that usually seen in exudative syphilis. In fact, had it not been for the paretic gold curve, we would not have hesitated to make a diagnosis of basilar meningitis. The rapid reversal of the gold curve towards the luetic type moreover confirms this diagnosis.

These three cases illustrate how well patients suffering from central nervous syphilis of the exudative type respond to general treatment. This response is manifest not only by clinical improvement, but by a rapid disappearance of the abnormal elements in the cerebrospinal fluid. Whether a paretic type of gold curve, as seen in Case 6, will alter the prognosis, is a question for the future to decide.

Group III.—Tabetic Type.

In *tabes dorsalis*, new problems confront us. It is probable from the history of many of these patients that even before the appearance of clinical symptoms, an inflammatory condition of the meninges has existed for years. It is only after sufficient number of tract or nerves have become involved to call forth symptoms that the patient's attention is directed towards the true nature of his disease. Certain cases seem to be spontaneously arrested and occasionally the cerebrospinal fluid is practically normal. In such patients there is often no further advance in clinical signs or symptoms. On the other hand, in the majority of patients, the condition of the cerebrospinal fluid points to an active inflammation, and the disease is progressive in nature. Because the active part of the disease manifests itself mostly in the cord and surrounding meninges, this condition is most favorable to local therapy applied by means of lumbar puncture. How-

ever, every patient does not require intraspinal therapy; many of them respond satisfactorily to general treatment.

TABLE VII.

J. F. Male. Age 64. Tabes Dorsalis. 10 years (?)
Syphilis (?)

DATE	BLOOD	CEREBROSPINAL FLUID			TREATMENT
	W. R.	Cells	Glob.	Wassermann Reaction	Salvarsan Intravenously
1914					
Oct. 28	++++	23	+	0.4 c.c. +++++	
31					0.3 gm.
Nov. 12	++++				0.35 gm.
28	++++				0.5 gm.
Dec. 12	++				0.4 gm.
29					0.4 gm.
1915					
Feb. 4					0.5 gm.
Mar. 20	++++	6	±	1.0 c.c. —	0.3 gm.
Apr. 16	+++				0.3 gm.
June 20	++++				0.3 gm.
Oct. 26	+++				0.3 gm.
Nov. 23	++++				0.3 gm.
1916					
May 17		4	—	2.0 c.c. —	0.3 gm.
June 7	++				0.3 gm.
30	±				0.3 gm.
July 21					0.3 gm.
1917					
Jan. 19	+				0.3 gm.
Feb. 16	+	20	±	2.0 c.c. +++	1112100000 Gold

Case 7 (J. F.) is an example of this response. He had suffered from "rheumatic pains" in the legs for over ten years, and was known to have had sluggishly reacting pupils, and diminished knee jerks in 1904. In 1912 he obtained considerable relief following a course of mercury injections. For four months he has had vertigo, weakness, and numbness in the left arm and both feet. On examination he showed Argyll-Robertson pupils, slight deafness in left ear, a band of hypoaesthesia and hypoalgesia about the chest; deep pain sensation lost in tendo Achilles and in right testicle; slight ataxia in

legs; slight Romberg; triceps, patellar and Achilles reflexes absent. The cerebrospinal fluid indicated an active meningitis. He was easily poisoned by salvarsan in large doses or when too frequently repeated, nevertheless, moderate treatment soon brought the cerebrospinal fluid to normal. Because of the patient's sensitiveness to salvarsan, subsequent injections have been given at longer intervals. In January, 1917, after an interval of six months without treatments, there was some return of the pains and an increase of tingling and numbness of the left arm and leg. A physical examination showed no objective increase in the signs of tabes, but an examination of the cerebrospinal fluid indicated a return of the meningitis. Even though the abnormal elements were not so marked as at the time of the original examination, they indicated that the disease was still present in the cerebrospinal axis, and hence treatment has been resumed. The patient, however, has been able to resume his work which previous to treatment he could attend to only occasionally.

While in some cases general treatment alone may be sufficient completely to arrest the progress of the disease both from the clinical and laboratory point of view, in other cases some additional form of therapy seems to be required.

Case 8 (G. M.) is an example of this. Patient's symptoms of tabes began in 1914 at which time he received salicylate of mercury and neosalvarsan. Upon admission to the Presbyterian Hospital Dispensary in 1915, he showed signs of an early tabes. Under mercury intramuscularly and iodides there was evidently no advance in the symptoms, until suddenly in the early part of 1916 when a Charcot's knee joint appeared. Upon admission to the hospital, he showed incomplete Argyll-Robertson pupils, hypotonia of the knees and hips, effusion into the right knee joint, hypoaesthesia in both legs, pain conduction delayed in the legs, deep pain sensation much diminished in the right tendo Achilles, but present in the left. Sense of position lost in the ankles and toes. Some incoordination in legs; deep reflexes present in arms and legs. His cerebrospinal fluid contained only four cells to the cubic millimeter, but the Wassermann reaction was as intense as one year previously. After nine month's treatment with salvarsan the Wassermann reaction is present in the same strength, and there is an increase of pleocytosis. There has been no

TABLE VIII.

G. M. Male. Age 35. Tabes Dorsalis. Duration (?).

Tabetic arthropathy, 2 mos. Syphilis, 11 yrs.

DATE	BLOOD	CEREBROSPINAL FLUID				TREATMENT		
	W. R.	Cells	Glob.	Wassermann Reaction	Gold	Sal. Intrav.	KI	Hg.
1914						Neo. 4 inj.	KI	
1915 Mar.	++++	23	-	0.6 c.c. ++++	♦		"	
Apr. to	++++						"	Hg. Sal. 2 gm.
1916							"	30 hypo.
Mar.							"	
Mar. 4	0	4	+	0.6 c.c. ++++				
18	0					0.35 gm.		
31	+					0.3		
Apr. 17	+					0.4		
May 8	0					0.4		
23	++					0.4		
June 6						0.25		
20	++	7	+	1.0 c.c. ++++		0.3		
July 12	0					0.3		
26	0					0.3		
Aug. 8	0					0.3		
24	+					0.33		
Sept. 9	++					0.3		
23	++					0.2		
Oct. 7	++					0.4		
Nov. 4	++					0.25		
18					11122	0.3		
Dec. 2	+	29	=	0.6 c.c. ++++	10000			

advance in symptoms, and as a brace has been applied to the leg, the arthropathy is no more marked. It is evident, however, that the active inflammatory condition is not subsiding.

It was because of a similar lack in response to general therapy that we* originally sought some additional form of treatment. As it had previously been shown that the serum of salvarsan treated patients had some general therapeutic effect, it was decided to determine whether intraspinal injections of this serum would be beneficial in a

*Swift, Homer F., and Ellis, A. W. M.: Jour. Am. Med. Assn., 1911, lvii, 2051; Arch. Int. Med., 1913, xii, 311.

condition like tabes dorsalis. In order to submit this theory to the severest test the patient was first treated only intraspinally.

TABLE IX.

C. H. L. Age 50. Tabes Dorsalis, 10 years.
Syphilis, 30 years.

DATE	BLOOD	CEREBROSPINAL FLUID			TREATMENT	
	W. R.	Cells per c.mm.	No-guchi Glob.	Wassermann Reaction	Intravenous	Intraspin. . Other Patients' Serum
1911						
Oct. 25	—	42	+++	0.2 c.c. +++++		
Oct. 30		41	++	0.2 c.c. +++++		25 c.c. of 40%
Nov. 6		40	++	0.2 c.c. +++++		20 c.c. of 40%
Nov. 18		50	++	0.4 c.c. +++++		22 c.c. of 40%
Dec. 3	—	18	++	0.4 c.c. +++++		26 c.c. of 50%
Dec. 12		17	++	0.2 c.c. +++++		30 c.c. of 50%
Dec. 28		15	++	0.8 c.c. +++++		25 c.c. of 40%
1912						
Jan. 6		15	++	0.2 c.c. +++++		25 c.c. of 40%
Jan. 12		10	++	0.4 c.c. +++++		30 c.c. of 50%
Jan. 23		14	++	1.0 c.c. =		26 c.c. of 50%
Jan. 31		16	+	1.0 c.c. +		30 c.c. of 50%
Feb. 7		17	+	1.0 c.c. ++		30 c.c. of 50%
Mar. 4	—	15	+	1.0 c.c. ++		
Apr. 10		10	=	1.0 c.c. +		
Apr. 16 to	—				Salvarsan 5 × 0.3 gm.	
May 14	—					
May 14		19	+	1.0 c.c. +		
Sept. 24	—	8	=	1.0 c.c. —		
1913						
Mar. 28	—	5	—	1.0 c.c. —		
June 21		2	—	1.0 c.c. —		
1914						
July 19	—	3	—	2.0 c.c. —		
1915						
July 3	—	3	=	2.0 c.c. —		

Case 9 (C. H. L.) is an example of the effects which may be secured from the intraspinal injection of serum alone. The patient's syphilitic infection occurred thirty years previously. He had had symptoms of tabes, such as lightening pains and numbness in the legs for the past ten years, and for six months previous to treatment had

vomited daily. On physical examination, he showed evidences of a fairly advanced tabes with marked superficial and deep sensory disturbances, Argyll-Robertson pupils, diminished hearing and absence of knee jerks, one ankle jerk and much diminished tendon reflexes in arms. The treatment causes a steady diminution in all the abnormal elements in the cerebrospinal fluid. When the fluid was almost normal, five intravenous injections of salvarsan were given and then treatment was discontinued. He returned to his work and for three years the cerebrospinal fluid has remained normal. Because of similar results in several tabetics, we felt that beneficial effects were to be expected from intraspinal injections of autosalvarsanized serum.

The question naturally arose as to whether intraspinal injections of normal serum might not have a similar effect. This was tested in several patients in some of whom it was found that the abnormal elements in the cerebrospinal fluid would disappear under intraspinal injections of normal serum alone. It then remained to be determined whether salvarsanized serum was more potent than normal serum. The results of such an investigation are shown in Case 10 (J. H. M.).

This patient had chancroid fourteen years before admission. No secondaries. Two and one-half years ago, numbness in fingers and toes, Argyll-Robertson pupils, absence of knee jerks. During two previous years patient had received two courses of mercury injections and much potassium iodide, as well as three injections of salvarsan intravenously. On admission he had Argyll-Robertson pupils, diminished hearing, slight patchy hyperalgesia, slightly ataxic gait, definite Romberg, sluggish deep reflexes in arms, and absence of deep reflexes in legs. After seven intraspinal injections, of 50 per cent normal serum, the cell count dropped to between 20 and 25, the number of cells remaining practically the same during the latter half of this course of treatment. The globulin remained the same, the Wassermann reaction was only slightly weaker. After eight injections of salvarsanized serum obtained from other patients, the cells dropped to about 10 to the cubic millimeter. Here the drop occurred shortly after the institution of salvarsanized serum injections; the globulin remained the same, but the Wassermann reaction decreased to half its original strength. The institution of combined intravenous injections of salvarsan, and intraspinal injections of patient's own serum,

TABLE X.

J. H. M. Age 44. Tabes Dorsalis, 2½ years. Syphilis, 14 years.

DATE	BLOOD	CEREBROSPINAL FLUID			TREATMENT	
	W. R.	Cells per c.mm.	Wassermann Reaction		Intravenous.	Intraspinal
1914						
Jan. 27	++++	100	+ 0.2 c.c. +++++			
Feb. 2	++++	69	+ 0.4 c.c. +++++			30 c.c. of 50% Normal Serum
Feb. 16	+++	20	= 0.6 c.c. +++++			30 c.c. of 50% " "
Mar. 2	++++	25	+ 0.4 c.c. +++++			30 c.c. of 50% " "
Mar. 16	++++	14	+ 0.4 c.c. +++++			30 c.c. of 50% " "
Mar. 30	++++	25	+ 0.4 c.c. +++++			30 c.c. of 50% " "
Apr. 13	++++	23	= 0.6 c.c. +++++			30 c.c. of 50% " "
Apr. 29		22	+ 0.4 c.c. +++++			30 c.c. of 50% " "
May 29	+++	43	+ 0.4 c.c. +++++			30 c.c. of 50% Sal. Serum
June 1	+++	18	+ 0.6 c.c. +++++			30 c.c. of 50% " "
June 22	+++	10	+ 0.8 c.c. +++++			30 c.c. of 50% " "
July 6	+++	10	+ 0.6 c.c. +++++			40 c.c. of 50% " "
July 20	+++	8	+ 0.8 c.c. +++++			38 c.c. of 50% " "
Aug. 3	+++	14	+ 2.0 c.c. +++++			40 c.c. of 50% " "
Aug. 24	+++	20	+ 1.0 c.c. +++++			40 c.c. of 50% " "
Sept. 7	+++	10	+ 0.8 c.c. +++++			40 c.c. of 50% " "
Oct. 12	++++	3	+ 0.8 c.c. +++++	Sal. 0.5 gm.	40 c.c. of 50%	Own Serum
Oct. 26	++++	10	+ 1.0 c.c. +++++	" 0.5 gm.	40 c.c. of 50%	" "
Nov. 9	++++	5	= 1.0 c.c. +++++	" 0.5 gm.	40 c.c. of 50%	" "
Dec. 7	++	5	+ 1.0 c.c. +++++	" 0.5 gm.	40 c.c. of 50%	" "
Dec. 21	++++	4	- 1.0 c.c. +++++	" 0.5 gm.	40 c.c. of 50%	" "
1915						
Jan. 4	++++	3	+ 0.8 c.c. +++++	" 0.5 gm.	40 c.c. of 50%	" "
Jan. 18	++++	3	= 0.8 c.c. +++++	" 0.5 gm.	40 c.c. of 50%	" "
Feb. 1	++++	3	= 1.0 c.c. +++++	" 0.5 gm.	40 c.c. of 50%	" "
Feb. 15	++++	4	- 1.0 c.c. +++++	" 0.5 gm.	40 c.c. of 50%	" "
Mar. 1	++	3	+ 0.8 c.c. +++++	" 0.5 gm.	40 c.c. of 50%	" "
Apr. 26	++++	12	+ 0.6 c.c. +++++	" 0.5 gm.	40 c.c. of 50%	" "
May 10	++++	1	= 0.4 c.c. +++++	" 0.5 gm.	40 c.c. of 50%	" "
May 24	+	2	+ 0.8 c.c. +++++	" 0.5 gm.	40 c.c. of 50%	" "
June 21	++	3	= 2.0 c.c. +	" 0.5 gm.	40 c.c. of 50%	" "
July 7		4	+ 2.0 c.c. -			

caused a further drop of cells to normal, and a weakening of the globulin and the Wassermann reaction. It required, however, fourteen injections of each before the reaction in the spinal fluid became nega-

tive. The increase in the pleocytosis and Wassermann reaction while the treatment was discontinued between March 1 and April 14, 1915, is noteworthy. The renewal of treatment, however, led to rapid diminution in the strength of the Wassermann reaction in the fluid as well as in the blood serum. The result in this case seemed to indicate that salvarsanized serum is more potent than normal serum, and that the combined intravenous and intraspinal treatments are more active than either form alone.

After it was established that intraspinal injections alone were of benefit, it was desirable to determine whether the combined form of treatment was more effectual than the intravenous alone. In Case 11, this fact seems to be established. Case 11 (C. L. V.)* was admitted January 10, 1911, with a history of a genital ulcer sixteen years previously. For past six years increasing lightening pains in legs, in the past two years loss of sense of position in feet and legs and increasing ataxia; nine months ago developed a Charcot hip. On admission patient had incomplete Argyll-Robertson pupils, very ataxic gait, hypotonia of hips and marked hypoaesthesia over lower chest and upper abdomen, entire loss of sense of position in lower extremities, deep reflexes present in arms, absent in legs. At the time of admission he was so weak and ataxic that he required two canes as well as the assistance of some other person in order to walk. Even though under the influence of mercury, he showed 125 cells and a heavy globulin in the spinal fluid. After the first three injections of salvarsan there was distinct subjective improvement and a gain in weight; the pleocytosis, however, was more marked than in the beginning, the globulin less. Mixed treatment by mouth reduced the number of cells, and further salvarsan treatment caused the cells to drop to 26. Unfortunately, during the first eight months of observation only the smaller quantities of cerebrospinal fluid were used for the Wassermann reaction, hence the cell count and globulin are the best criteria for judgment of the effects of treatment at this time. After the course of salvarsan was discontinued six weeks, the number of cells had risen to 50. The addition of intraspinal to intravenous

* In this and subsequent cases the intraspinal treatments consisted of injections of the patients' own serum obtained in one-half to one hour after intravenous injections of salvarsan.

TABLE XI.

C. L. V. Age 35. Tabes Dorsalis, 6 years. Syphilis, 16 years.

DATE	BLOOD	CEREBROSPINAL FLUID			TREATMENT	
	W. R.	Cells per c.mm.	Noguchi Glob.	Wassermann Reaction	Intravenous	Intraspinal Serum
1910						
Nov. 10 to 1911	++++				16 injections HgCl ₂ intramuscularly	
Jan. 10	++					
Jan. 16	+	125	++	0.2 c.c. -	Salvarsan 0.2 gm.	
25	++				" 0.3 gm.	
Feb. 8	++				" 0.36 gm.	
13		150	=	0.2 c.c. -		
Feb. 15 to Aug. 20	+				Mixed treatment by mouth	
Aug. 21	+	63	+	0.2 c.c. =?		
Aug. 26 to Sept. 22	=				Salvarsan 5 × 0.2 gm.	
22		26	=	0.2 c.c. -		
Nov. 24					" 0.2 gm.	
Dec. 1	=	50	+	1.0 c.c. +	" 0.2 gm.	18 c.c. of 50%
Dec. 11	=	20	+		" 0.2 gm.	30 c.c. of 50%
Dec. 18	=	20	=	1.0 c.c. +	" 0.2 gm.	20 c.c. of 40%
1912						
Jan. 5	-	12	=	1.0 c.c. +++	" 0.3 gm.	30 c.c. of 40%
12	-	4	-	1.0 c.c. +	" 0.3 gm.	30 c.c. of 50%
15	-				" 0.2 gm.	
20		5		1.0 c.c. =?		
July 9	-	2	-	1.0 c.c. -		
Sept. 10	-	1	-	1.0 c.c. -		
May 15	-	3	-	1.0 c.c. -		
1914		Not counted				
Feb. 2	-		=	2.0 c.c. -		
Aug. 1	-	3	-	2.0 c.c. -		
1915						
June 24	-	1	-	1.0 c.c. -*		

* Not tested with 2 c.c.

treatment cause a rapid approximation to normal in the cerebrospinal fluid. At this time the patient also stated that subjectively his improvement had been more marked than at any previous time. For three and one-half years without treatment the cerebrospinal

fluid remained normal and there was no advance in the patient's tabetic symptoms.

The importance of treating a patient with tabes until the cerebrospinal fluid becomes normal and remains so is well illustrated in Case 12 (O. F.). Ten years before admission he had an indurated genital ulcer but no secondaries. At this time mercury pills were administered for three months. Four years ago he was injured in a football game, six months later lightening pains in legs appeared and have continued. Pains in chest for one year. Two years ago band-like sensation below knee. Difficulty in walking for three years. Has instructed himself in Fraenkel movements with considerable improvement in walking. On admission, pupils showed only slight reaction to light; there was marked ataxic gait, marked Romberg, loss of sense of position in lower extremities, prolonged pain sensation below knees, hypoaesthesia over trunk below the second intercostal space, superficial and deep reflexes all absent. When first seen this patient presented the signs of an extensive degeneration of the cord. During the first six months, he received repeated small intravenous injections of salvarsan with some clinical improvement, but only slight alteration in the cerebrospinal fluid. This consisted in somewhat decreased pleocytosis and decreased globulin, the Wassermann reaction remaining about the same strength. During 1912, he would report only occasionally for treatment and discontinued it altogether in the last three months of that year. At the end of this period he suffered a clinical relapse consisting of partial deafness, diplopia, and marked increase in pain. The cerebrospinal fluid showed an increased pleocytosis and increased strength of the Wassermann reaction. From this time on he faithfully continued treatment, which consisted of combined intravenous and intraspinal injections given in courses of six to eight treatments followed by free intervals of four to six weeks. Although the improvement in the condition of the cerebrospinal fluid was slow under this treatment, it was continuous and after a year the only abnormality was a weak Wassermann reaction in 2 c.c. quantities. Because of persisting positive reaction in the blood, another course of salvarsan was given intravenously.

The behavior of the Wassermann reaction in the blood serum was of interest. During the first year of the disease the reaction de-

TABLE XII.

O. F. Age 32. Tabes Dorsalis, $3\frac{1}{2}$ years. Syphilis, 10 years.
Improvement, relapse, improvement.

DATE	BLOOD		CEREBROSPINAL FLUID			TREATMENT	
	W. R.	Cells per c.mm. Noguchi Glob.	Wassermann Reaction			Intravenous	Intraspinal Serum
			Liver Antigen	Cholesterin Heart Antigen			
1911							
Apr. 4	++++	27	++	0.2 c.c. -		Salvarsan	
May 18	++					5×0.2 gm.	
19		39	++	0.2 c.c. ++			
Oct. 13	++					Sal. 0.2 gm.	
Oct. 20	+	9	+	0.2 c.c. +		" 0.2 gm.	17 c.c. of 40%
Oct. 27 to						" 4×0.2 gm.	
Nov. 17							
Nov. 20		10	+				
1912							
Apr. 9	-	14	=	0.4 c.c. +++++		" 0.3 gm.	30 c.c. of 40%
15						" 0.3 gm.	
23	+	3	+	0.4 c.c. +++++		" 0.3 gm.	30 c.c. of 40%
30	+					" 0.3 gm.	
May 7	+	6	+	0.4 c.c. +++++		" 0.3 gm.	30 c.c. of 40%
Sept. 23	+	19	+	0.6 c.c. +++++		Neo. 0.9 gm.	
Oct. 8	+					" 0.9 gm.	
	Chol. heart Anti.						
1913							
Jan. 31	+++	70	+	0.4 c.c. +++++	0.2 c.c. +++++	" 0.75 gm.	
Feb. 7	+++	57	=	0.4 c.c. +++++	0.4 c.c. +++++	" 0.9 gm.	30 c.c. of 40%
14	+++	28	=	0.6 c.c. +++++	0.4 c.c. +++++	" 0.9 gm.	30 c.c. of 40%
28	++++	17	=	0.6 c.c. +++++	0.4 c.c. +++++	" 0.9 gm.	30 c.c. of 40%
Mar. 14	++	14	-	0.4 c.c. +++++	0.4 c.c. +++++	" 0.75 gm.	30 c.c. of 40%
27	++++	7	+	1.0 c.c. +++++	0.6 c.c. +++++	" 0.75 gm.	30 c.c. of 40%
Apr. 9	+++	7	+	1.0 c.c. +	0.6 c.c. +++++	" 0.9 gm.	30 c.c. of 40%
June 3	++++	5	=	1.0 c.c. ++	0.8 c.c. +++++	Sal. 0.5 gm.	30 c.c. of 40%
June 12	++++					Neo. 0.9 gm.	
17	++++	2	-	1.0 c.c. =	1.0 c.c. +++++	Sal. 0.5 gm.	30 c.c. of 40%
July 2		4	-			" 0.5 gm.	30 c.c. of 40%
15	+++	2	-		0.8 c.c. +++++	" 0.5 gm.	30 c.c. of 40%
29	+++	1	-		1.0 c.c. ++	" 0.5 gm.	30 c.c. of 40%
Aug. 12	+	3	-		0.8 c.c. ++	" 0.5 gm.	30 c.c. of 40%
Sept. 9	+±					" 0.5 gm.	
23	--	2	=	1.0 c.c. -	1.0 c.c. +++++	" 0.5 gm.	30 c.c. of 50%

TABLE XII—*Continued.*

DATE	BLOOD		CEREBROSPINAL FLUID			TREATMENT	
	W. R.	Cells per c.mm.	Noguchi Glob.	Wassermann Reaction		Intravenous	Intraspinal Serum
				Liver Antigen	Cholesterin Heart Antigen		
1913							
Oct. 7	--	3	—	2.0 c.c. ±	2.0 c.c. + + + +	Neo. 0.75 gm.	30 c.c. of 50%
21.	± ±					" 0.75 gm.	
Nov. 17						Sal. 0.5 gm.	
Dec. 1	++++					" 0.5 gm.	
22	++++					" 0.6 gm.	
1914							
Jan. 19		3	—	2.0 c.c. —	2.0 c.c. + +		
27	++						
Mar. 16	++					" 0.5 gm.	
Mar. 30	+++					" 0.5 gm.	
Apr. 13	—					" 0.5 gm.	
Apr. 30	±?					" 0.5 gm.	
May 28	—					" 0.5 gm.	
June 12	—	3	—		2.0 c.c. —		
1915							
Feb. 27	—	4	—		2.0 c.c. —		

creased steadily. However, it was only tested with a liver antigen. With cholesterinized antigen a strongly positive reaction continued until September, 1913. Upon the resumption of salvarsan treatment in November of this year, the reaction became strong again, after a month without treatment it was weaker, but again showed a strong provocative phase after which it became completely negative and remained so. When the patient was first seen although the clinical findings indicated a very advanced tabes, the cerebrospinal fluid findings indicated an active meningitis. Under prolonged and intermittent treatment, the laboratory evidence of active disease disappeared and there was no advance in the tabetic process except possibly the development of complete Argyll-Robertson pupils. The clinical relapse in January, 1913, with a simultaneous increase in pleocytosis and in the Wassermann reaction is worthy of special notice and shows the importance of treating this patient until the cerebrospinal fluid

became normal. The addition of the intraspinal to the intravenous treatment seems to have been especially advantageous. The fact that at the end of treatment all laboratory findings were negative and have not recurred indicates a cure.

TABLE XIII.

F. H. H. Age 39. Tabes Dorsalis, 4 years? Syphilis, 12 years.

DATE	BLOOD	CEREBROSPINAL FLUID			TREATMENT	
	W. R.	Cells per c.mm.	No-guchi Glob.	Wassermann Reaction	Intravenous	Intraspinal Serum
1913						
Mar. 18	=	24	=	0.2 c.c. +++++		
20	+	42	=	0.2 c.c. +++++	Sal. 0.4 gm.	30 c.c. of 40%
Apr. 17	++	9	=	0.4 c.c. +++++	" 0.5 gm.	30 c.c. of 40%
May 13	+	2	=	0.4 c.c. +++++	" 0.5 gm.	30 c.c. of 40%
June 3	+		=	0.4 c.c. +++++	" 0.5 gm.	30 c.c. of 40%
19	+	5	+	0.4 c.c. +++++	" 0.5 gm.	30 c.c. of 40%
July 7	=	2	+	0.4 c.c. +++++	" 0.5 gm.	30 c.c. of 40%
Sept. 19	-	2	-	0.6 c.c. +++++	" 0.5 gm.	30 c.c. of 40%
Oct. 10	-	3	=	0.6 c.c. +++++	" 0.5 gm.	30 c.c. of 50%
24	-	2	=	0.4 c.c. +++++	" 0.5 gm.	30 c.c. of 50%
Nov. 21	-	2	=?	0.8 c.c. +++++	" 0.5 gm.	30 c.c. of 50%
Dec. 12	-	2		0.6 c.c. +++++	" 0.5 gm.	30 c.c. of 50%
1914						
Jan. 16	-	1	-	0.6 c.c. +++++	" 0.4 gm.	
Feb. 6	-	1	-	0.6 c.c. +++++	" 0.5 gm.	30 c.c. of 50%
27	-	2	=	0.4 c.c. ++	" 0.5 gm.	30 c.c. of 50%
Apr. 3		1			" 0.5 gm.	40 c.c. of 50%
May 4	-	1	=	2.0 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
June 5	-	1		1.0 c.c. +++++		40 c.c. of 50%
July 10	-	4	+	2.0 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
24	-	0	-	2.0 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
Aug. 7	-	1	=?	2.0 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
Sept. 4	-	2	-	2.0 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
1915						
June 17	-	2	-	2.0 c.c. -		

While the discontinuing of treatment may at times be followed by an increase in the abnormal elements in the cerebrospinal fluid, the contrary is not infrequently seen. This leads us to speak of the importance of giving treatment in courses with periods of rest and of

determining what has occurred during these free intervals. Case 13 (F. H. H.) illustrates this point. Twelve years before admission, he had a chancre followed by a rash. He took KI and pills for two and one-half years and inunctions for two months, then four injections of mercury yearly for six years. Four years ago began to tire easily and had dull pains shooting up and down the spine. Six months later he developed weakness in legs and difficulty in walking. He then received thirty-seven injections of mercury with relief from pain. Two years ago had slight numbness in arms and legs. In January, 1911, two injections of salvarsan; July, 1912, lumbar puncture—66 cells, globulin increased, Wassermann reaction strongly positive, amount not given. September 16 to October 7, 1912, neosalvarsan. Inunctions of mercury for the past month. Condition on admission: Irregular pupils, reaction slowly to light; sensation normal; absence of tendo Achilles and patellar reflexes; no mental disturbance. Because of the markedly abnormal condition of the cerebrospinal fluid, even though the patient was under the influence of fairly active treatment, it was considered advisable to give combined intraspinal and intravenous injections immediately. There was a prompt diminution in pleocytosis and a steady weakening of the Wassermann reaction. When treatment was intermitted in July, 1913, the fluid continued to show improvement. Slight increase in the intensity of the Wassermann reaction occurred when the treatment was resumed. Treatment was again intermitted in February, 1914, the Wassermann reaction became much weaker and remained of practically the same strength under irregular treatment until September. Treatment was then stopped and a year later the cerebrospinal fluid was normal. There has been no advance in the clinical symptoms. As the patient lives in a distant city, we have been unable to examine the fluid lately.

Group IV.—Paralytic Dementia.

When compared with the rate of diminution of the abnormal elements in the spinal fluid and the arrest of clinical symptoms and signs in tabes, the treatment of paresis has been a distinct disappointment. When compared with the results of our therapeutic efforts in the presalvarsan era, however, treatment is encouraging and

distinctly worth while. The results reported by different observers vary widely. These differences are no doubt explainable in the class of patients who come under observation and treatment. As might be expected in a disease which involves the parenchymatous tissue so extensively, the earlier the treatment is instituted, the better the chance of improvement. If, on the other hand, the condition has progressed to such an extent that it is necessary to commit the patient to an institution, the chances for improvement are diminished. The increasing difficulty in favorably influencing the disease as it grows older is illustrated in Case 14.

Case 14 (R. P.). He was first seen March 31, 1913. Denied primary or secondary syphilitic lesion. In 1903 he had a sore throat which was suspected of being syphilitic in nature. Has had stiffness in arms and legs for ten years. Has had tremor of the hands for four years, and shooting pains in various parts of the body. Four months ago following a fall he had weakness of the muscles of the left leg and foot. This gradually improved. Patient feels that his mental acuity is diminishing and his memory is poor especially for names of people. He is much worried over business affairs. His physical examination showed small pupils reacting with only slight excursion to light. Coarse tremor of tongue, arms and legs, slight incoordination in legs. No sensory disturbances. All tendon reflexes are overactive except the tendo Achilles which are absent. Mentally, patient was nervous and depressed, but well oriented. The first course of treatment which was given in a fairly early period in the disease, resulted in a rapid diminution of both pleocytosis and globulin, and in the strength of the Wassermann reaction. He then discontinued treatment, but six months later was persuaded to return for another course. At this time there was no increase in cells, but the Wassermann reaction was stronger. Again, as in the first course, treatment favorably influenced the laboratory findings, and again the patient discontinued his treatment voluntarily. He was, however, able to resume his work in a very responsible position. In the fall of 1915, he suffered a sudden attack of mental depression and for a time had to be confined in an institution. Treatment which was again resumed at this time had practically no effect on the strength of the Wassermann reaction in the spinal fluid. Several gold tests at this

TABLE XIV.
R. P. Age 43. Tabo-paresis.

DATE	BLOOD	CEREBROSPINAL FLUID				TREATMENT	
	W. R.	Cells	Glob.	Wassermann Reaction	Gold	Salvarsan	Intraspinal Serum
1913							
Apr. 7	++++	47	++	0.4 c.c. +++++		0.4 gm.	30 c.c. - 40%
22	++++	7	+	0.6 c.c. +++++		0.5 gm.	30 c.c. - 40%
May 6	++++	11	+	0.8 c.c. +++++		0.5 gm.	30 c.c. - 40%
20	++++	13	=	0.6 c.c. +++++		0.5 gm.	30 c.c. - 40%
June 3	++++	9	+	0.6 c.c. +++++		0.5 gm.	30 c.c. - 40%
20	++++	14	+	1.0 c.c. +++++		0.5 gm.	30 c.c. - 40%
1914							
Jan. 16	++++	3	=	0.6 c.c. +++++		0.5 gm.	
23	++++	4	=	0.6 c.c. +++++		0.5 gm.	40 c.c. - 50%
Feb. 5	++++	6	=			0.5 gm.	40 c.c. - 50%
23	++++	6	=	0.8 c.c. +++++		0.5 gm.	34 c.c. - 50%
Mar. 10	++++	6	=	0.6 c.c. +++++		0.6 gm.	40 c.c. - 50%
23	++++	5	=	0.8 c.c. +++++		0.5 gm.	40 c.c. - 50%
Apr. 6		3	-	1.0 c.c. +++++		0.5 gm.	40 c.c. - 50%
20	++++					0.5 gm.	
27	++++					0.5 gm.	
1915							
Dec. 11	++++	16	+	0.6 c.c. +++++		0.4 gm.	14 c.c. - 100%
20	++++	42	+	0.6 c.c. +++++		0.3 gm.	
30	++++		+	0.6 c.c. +++++		0.4 gm.	15 c.c. - 100%
1916							
Jan. 7	++++	8	=	0.2 c.c. +++++		0.4 gm.	15 c.c. - 100%
29	++++	5	=	0.2 c.c. +++++		0.5 gm.	15 c.c. - 100%
Feb. 12	++++	2	=	0.6 c.c. +++++		0.5 gm.	15 c.c. - 100%
25	++++	5	=	0.6 c.c. +++++		0.5 gm.	14 c.c. - 100%
Mar. 11	++++	9	=	0.6 c.c. +++++		0.3 gm.	15 c.c. - 100%
25	++++	10	=	0.6 c.c. +++++		0.3 gm.	15 c.c. - 100%
Apr. 7	++++	9	=	0.6 c.c. +++++		0.35 gm.	15 c.c. - 100%
May 6	++++	4	+	0.6 c.c. +++++		0.4 gm.	15 c.c. - 100%

time showed constantly the paretic type. Patient has again refused to be treated and while on superficial examination he appears fairly normal, still his mental condition is such that he can not return to his former work.

The diminishing influence of treatment as the disease progresses is still more strikingly brought out in Case 15 (G. W. C.). On admission, July, 1913, he complained of difficulty in thinking and of

aching sensation in the leg which had started seven months previously. A diagnosis of neurasthenia had been made. He was occasionally at loss for a word. The examination showed unequal, dilated pupils, right reacted only slightly to light, left not at all; diminished hearing, no Romberg, no incoordination, sensations normal, deep reflexes sluggish. When patient was admitted provisional diagnosis was paresis, but the symptoms were mainly neurasthenic in character; the later symptoms, however, confirmed the original diagnosis. During the first course of treatment, there was a rapid diminution in pleocytosis, and in the Wassermann reaction in the spinal fluid, so that after four months it required 2 c.c. of fluid to give a positive reaction. After patient had been under treatment for nine months, he discontinued it for three months. At the end of this interval, there was a definite clinical relapse, patient showing distinct mental exaltation. The Wassermann reaction in the blood had become strongly positive; the spinal fluid showed 77 cells and a positive Wassermann reaction. After two months' treatment there was again a definite drop in the cell count but not so marked as with the first course. The Wassermann reaction responded more slowly. Again patient discontinued treatment. After an interval he returned with a relapse to 90 cells in the fluid, the Wassermann reaction remained of the same strength. After nine treatments the cells dropped to 10, the globulin remained unchanged, and the Wassermann reaction showed no decrease in intensity. However, the patient was somewhat improved clinically. After another interval of three months without treatment another clinical relapse occurred as shown by speech disturbance, slightly diminished capacity for work and definite euphoria. At this time there were 73 cells, globulin reaction stronger, the Wassermann reaction the same intensity. After July, 1915, the treatment was given once or twice every month, but in spite of this there was a progressive downward course in the clinical condition and the cerebrospinal fluid was practically uninfluenced. There were increasing speech disturbance, increased difficulties in writing, weakness in the right leg, occasional feeling of numbness and pricking in the forearm and right leg. Patient attempted to continue his business, but was less capable than formerly. It was never thought necessary to commit him to an institution, and he died

TABLE XV.

G. W. C. Age 44. Paresis. Syphilis, Duration?

DATE	BLOOD	CEREBROSPINAL FLUID			TREATMENT	
	W. R.	Cells	No-guchi Glob.	Wassermann Reaction	Intravenous	Intraspinal Serum
1913						
July 17	++++	34	+	0.6 c.c. +++++		
22	++++				Sal. 0.5 gm.	
29	+++	30	++	0.8 c.c. +++++	" 0.4 gm.	30 c.c. of 40%
Aug. 10	++++				" 0.5 gm.	
18	+++				" 0.5 gm.	
26	+++				" 0.5 gm.	
Sept. 17	+++	5		1.0 c.c. —	Neo. 0.9 gm.	30 c.c. of 50%
Oct. 1	++++	7	+	2.0 c.c. +++	" 0.9 gm.	30 c.c. of 50%
Nov. 13	++++	8	+	2.0 c.c. ++	" 0.5 gm.	30 c.c. of 66%
Nov. 28	++++				" 0.1 gm.	
Dec. 5 to						
Mar. 27/14					Sal. 9×0.5 gm.	
June 29	++++	77	+	0.6 c.c. +++++	" 0.5 gm.	
July 1	++++	65	+	0.6 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
20	+++	57	+	0.6 c.c. +++++	" 0.5 gm.	35 c.c. of 50%
Aug. 3	++	22	+	2.0 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
10	+++				" 0.5 gm.	
20	+++	22	+	2.0 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
Sept. 4	++	15	+	0.8 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
Nov. 23	++++	90	+	0.8 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
Dec. 7	++	36	+	0.8 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
21	++	14	+	0.6 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
1915						
Jan. 19	++	20	+	0.6 c.c. +++++	" 0.5 gm.	35 c.c. of 50%
Feb. 15		32	+	0.6 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
Mar. 1	++	12	+	0.6 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
15	++++	13	+	0.6 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
27		12	+	0.8 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
Apr. 12	++	10	+	0.6 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
July 20		73	++	0.8 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
Aug. 6		20	++		" 0.5 gm.	40 c.c. of 50%
18		20			" 0.5 gm.	40 c.c. of 50%
Sept. 9	+	28		1 c.c. +++++	" 0.5 gm.	
23		11		0.4 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
Oct. 11		20			" 0.5 gm.	40 c.c. of 50%
Nov. 9	+	25	=	0.6 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
22	++++	17		0.6 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
Dec. 6	++	15	+	0.8 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
22	=	16	+	0.8 c.c. +++++	" 0.5 gm.	40 c.c. of 50%

TABLE XV—*Concluded.*

DATE	BLOOD	CEREBROSPINAL FLUID			TREATMENT	
	W. R.	Cells	No-guchi Glob.	Wassermann Reaction	Intravenous	Intraspinal Serum
1916						
Jan. 1					" 0.5 gm.	
13					Neo. 0.6 gm.	
Feb. 2		20			Sal. 0.5 gm.	40 c.c. of 50%
18		17			" 0.5 gm.	40 c.c. of 50%
Mar. 3		7		0.6 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
Apr. 8				0.6 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
21	++++			0.6 c.c. +++++	" 0.5 gm.	40 c.c. of 50%
May 6					" 0.5 gm.	40 c.c. of 50%
June 28	++++	20		0.6 c.c. +++++	" 0.5 gm.	40 c.c. of 50%

August, 1916 while on a business trip. The gold curves during the last year of his life were all of strongly paretic type.

These two cases are fairly typical of what may be expected in the treatment of paresis. First, marked clinical improvement to the extent of complete remission with simultaneous improvement in the condition of the cerebrospinal fluid. Second, marked or complete remission with practically no improvement in the condition of the cerebrospinal fluid. Third, a stationary condition of the cerebrospinal fluid and a progressive downward course clinically, even though most intensive treatment is being applied. In no case can we accurately prognosticate as to which of these three types of response will occur. Most of the patients, late in the disease, show the tendency to a progressive downward course even under treatment.

What then is the effect of treatment in paresis? In the majority of patients who are treated at an early period in the disease, there is an increased number of remissions. As a consequence of this, there are longer periods of usefulness, and shorter periods of confinement in institutions. Several observers have stated that where formerly many of the paretics in the insane asylums were bedridden for long periods, now they are helpless for a much shorter time, and the patients are often socially possible until near the time of death.

Patients Clinically not Paralytica Dementia in Whom the Cerebrospinal Fluid Shows Paretic Type of Gold Curve.

With the application of the colloidal gold test to the cerebrospinal fluid of all patients with cerebrospinal involvement, we are occasionally finding a paretic type of gold curve in all stages of the disease; at least in all intervals after infection. During the past year we have had one patient who within less than a year from the time of his initial lesion, showed pronounced changes in the cerebrospinal fluid with a marked paretic type of gold curve. Patients with apparently an exudative lesion, such as Case 5, also have given this type of curve in their cerebrospinal fluids, but more frequently the paretic curve is found in the fluids of patients with clinical signs and symptoms of tabes dorsalis. The question naturally arises as to what influence the conditions which give rise to this peculiar type of gold curve may have upon the response to treatment. We have seen that when patients with early paresis give this type of curve that the influence of treatment may be slight and that the curve may be unaffected. On the other hand, a case like Case 5 with exudative lesions may show a rapid reversal of the paretic curve to a luetic curve.

The response of a tabetic with a paretic gold curve in his fluid to intensive treatment is illustrated in Case 16 (F. C.) who was admitted April 3, 1915. Infection fourteen years ago with rash. Treatment consisted of pills for one year, inunctions one month. Two years ago hot flashes down arms and legs recurring over periods of two or three days. Six months ago shooting pains in right leg, improvement after massage. Numbness in soles of feet for several months. Ten days ago return of the pain. For two years cold baths have caused him pain where formerly they were enjoyed. Examination showed Argyll-Robertson pupils, diminished hearing, hyperalgesia over front of chest, band of hyperesthesia to cold over lower trunk, pain sensation delayed over legs and lower trunk, deep sensations all retained, slight Romberg, no ataxia, arm reflexes exaggerated, knee jerks normal, ankle jerks much diminished. The first course of treatment was accompanied by a satisfactory diminution in pleocytosis, but there was no effect on the intensity of the Wassermann reaction in the fluid. After discontinuing treatment for four months, the fluid Wassermann

TABLE XVI.

F. C. Male. Age 52. Tabo-paresis? Syphilis, 14 years.
Lancinating pains, 2 years.

DATE	BLOOD	CEREBROSPINAL FLUID				TREATMENT		REACTION
	W. R.	Cells	Glob.	W. R. c.c.	Gold	Intrav. Sal. gm.	Intrasp. Serum 100%	
1915								
Apr. 5	++++	20	++	0.6	++++	55554 00000	0.3	
19	++++		++				0.3	
May 3	++++	6	++				0.4	15 c.c.
17		30	++	0.6	++++		0.35	15 c.c.
June 5	++++	8	++				0.4	15 c.c.
20	++++	2	++	0.6	++++		0.4	15 c.c.
Aug. 8		6	++	0.6	++++		0.4	13 c.c.
								Vomiting, headache, chill 6 hrs. p. IV
Dec. 5	++++	10	++	1.0	+++		0.4	17 c.c.
1916								
Jan. 15	++++	13	++	1.0	++++		0.35	16 c.c.
Feb. 12	++++	11	++	2.0	++++		0.25	15 c.c.
26	++++	8	+	2.0	++++		0.3	15 c.c.
Mar. 18	++++	6	=	2.0	++		0.3	15 c.c.
Apr. 8	++++	6	+	2.0	++++		0.4	15 c.c.
22	++++	20	+	2.0	++++		0.3	15 c.c.
								" " "
May 20	++	9	++	2.0	-	11221 00000	0.3	15 c.c.
								Urticaria only reaction
June 3	++++	20	++				0.3	15 c.c.
17	++++			2.0	++		0.3	15 c.c.
								" " "
July to Oct.						Hg. Sal. hypo		KI
Nov. 4	=	12	+	2.0	+	11111 00000		
25	=						0.2	
Dec. 9	+						0.2	
1917								
Jan. 6	+++						0.3	
								Urticaria
20	=	28	+	0.6	++++	11233 21000		

reaction was weaker. This is contrary to what is usually found in paresis. Upon resuming treatment, the reaction in the fluid rapidly diminished but the cells showed a tendency to remain above normal

in number. The patient has shown marked anaphylactic symptoms during intravenous injections followed by urticaria and dermatitis. During a course of mercury and iodide, the condition of the fluid remained the same except that the gold curve became weaker. Because of only the slight abnormality of the cerebrospinal fluid it was felt that resumption of intravenous treatment alone might be sufficient. The patient, however, has continued to exhibit marked hypersensitiveness to the salvarsan and after three injections has shown an increased pleocytosis, a stronger Wassermann reaction, and a more marked luetic type of gold curve in his fluid. He has, however, shown marked subjective improvement, but practically no change in the physical signs. The difficulty in maintaining the improvement in the cerebrospinal fluid and the original parietic type of gold curve suggests that this patient may follow a course similar to that illustrated in the two cases of paresis. Whether the active syphilis can be permanently eliminated only the future will answer.

The rapid rate at which the gold curve may be reversed and the other abnormal elements in the spinal fluid diminished is illustrated by Case 17 (B. J.) who was admitted August 30, 1916. He had gonorrhea twelve years ago, no history of primary or secondary syphilitic lesions. For past eight years attacks of epigastric pains, sharp in character, aggravated by eating, relieved by vomiting. For the past two months, vomiting and pain much worse, suprapubic pain during voiding, occasional incontinence of urine. Examination showed an emaciated man, vomiting almost constantly, bladder markedly distended and inflamed, pupils react sluggishly to light, reflexes normal, only disturbance in sensation are two small areas of hypoaesthesia over midscapula, and two areas of hyperaesthesia over anterior chest just external to nipples, and a small patch over left side of epigastrium. After treatment was started the vomiting diminished somewhat, but the attacks would occur two or three times a week. He remained in the hospital until November 4, with slow but steady improvement. Occasional attacks of vomiting. After leaving hospital vomiting became less frequent. There was marked constipation, occasional vomiting while straining at stool. The improvement in the condition of the cerebrospinal fluid has been steady in all respects with a reversal in the type of the gold curve to a luetic

TABLE XVII.

B. J. Male. Painter. Age 36. Tabes. Gastric Crises, 8 years.
Syphilis?

DATE	BLOOD		CEREBROSPINAL FLUID			TREATMENT			
	W. R.	Cells	Glob.	Wassermann Reaction	Gold	Intrav. Sal.	Intrasp. Serum	KI gm.	Hg.
1916									
Sept. 2	0	21	+	0.6 c.c. ++++	55554 21000			50	HgCL ₂ 8 × ½ gr. hypo.
" 2-21									
" 21		32	++	2.0 c.c. ++++	55554 42100		15 c.c. 100%		
Oct. 3	0	57	++	2.0 c.c. ++++		0.2 gm.	15 c.c. "		
11	0	21	++	1.0 c.c. ++++		0.35	12 c.c. "		
17	0	13	++	2.0 c.c. +		0.3	12 c.c. "		
26	0	15	++	2.0 c.c. +		0.2	15 c.c. "		
Nov. 3	0	17	-	2.0 c.c. ++		0.3	12 c.c. "		
16	0	11				0.3	16 c.c. "		
Dec. 13	0	8	+	2.0 c.c. -	22232 10000	0.3	12 c.c. "		
" 28	0	4	+	2.0 c.c. +++		0.35	15 c.c. "		
1917									
Jan. 11	0	8	+	2.0 c.c. +		0.3	15 c.c. "		
24		0		2.0 c.c. ±	11221 10000	0.25	15 c.c. "		

type which is also diminishing in strength. The intraspinal treatments were combined with intravenous treatments from the beginning because we felt that the paretic type of gold curve made the prognosis more grave. In fact, because of the constant association of paretic type of curve in paresis we feel that when this condition is present in the fluid of patients in whom other forms of cerebrospinal syphilis are thought to be present, the most intensive treatment should be applied from the beginning, and that our prognosis as to ultimate permanent arrest should be guarded. The exact influence of this condition in the cerebrospinal fluid is still a matter for investigation.

SUMMARY.

In this presentation the various factors considered in classifying the cases are: time since infection, clinical picture, laboratory findings, and response to treatment. The following groups are recognized:

1. Early Meningitis.

(a) Cases which respond readily to the general administration of salvarsan and mercury.

(b) Cases which respond more slowly to salvarsan intravenously, and tend to relapse when salvarsan is discontinued, or mercury is substituted.

(c) Cases which do not clear up under most intensive general treatment, but which respond satisfactorily to intraspinal treatment.

2. Later forms of central nervous syphilis of the exudative type. The abnormal elements in the cerebrospinal fluid usually disappear rapidly under general administration of iodides, mercury and salvarsan.

3. Tabes Dorsalis.

(a) Cases which show a rapid response to general treatment.

(b) Cases which show no improvement or very slow improvement under general treatment.

(c) Cases which show a satisfactory response to intraspinal treatment alone.

(d) Cases which have responded slowly to general treatment, but which respond more rapidly when intraspinal injections of "auto-salvarsanized serum" are given.

(e) Cases which relapse when treatment is discontinued.

(f) Cases which continue to improve when treatment is discontinued.

4. Paralytica Dementia.

(a) Cases with marked improvement in both clinical signs and the condition of the cerebrospinal fluid.

(b) Cases with marked clinical improvement but no change in the cerebrospinal fluid.

(c) Cases with progressive downward clinical course and stationary condition of the cerebrospinal fluid.

5. Patients clinically not paralytica dementia in whom the cerebrospinal fluid shows a paretic type of gold curve.

(a) Cases which respond rapidly to combined intravenous and intraspinal treatment.

(b) Cases which respond more slowly and show a decided tendency for the abnormal elements to recur when treatment is discontinued.

CONCLUSION.

Before undertaking the treatment of a patient with any form of cerebrospinal syphilis, it is important to determine what symptoms are due to inflammation or exudation and what are due to degeneration of tracts or cortex. It is also advisable to determine the intensity of the irritative condition as indicated by the cerebrospinal fluid. In general the lesions due to inflammation or exudation are much improved or eliminated by the general treatment of the patient. Those due to degeneration are little, if any, affected. Treatment should be directed not only towards the elimination of symptoms, but towards the elimination of the underlying process, namely, syphilis. In most patients with early meningitis and in those with what was formerly termed tertiary syphilis of the central nervous system, the symptoms due to exudation respond in a satisfactory manner to the general administration of salvarsan, mercury, and potassium iodide. Occasionally, a case is met in which intraspinal treatment seems to be necessary in order to eradicate completely the central nervous lesions. Likewise in tabes dorsalis, many cases respond satisfactorily to the general administration of salvarsan and mercury. On the other hand, in a considerable number of tabetics, the addition of intraspinal injections of serum to intravenous treatment with salvarsan seems to hasten the elimination of abnormal elements in the cerebrospinal fluid and lead to a permanent arrest of the degeneration. It is advisable to continue the treatment of patients suffering from cerebrospinal syphilis or tabes dorsalis until the cerebrospinal fluid is normal and remains so. A possible exception may be made in reference to excess globulin, for an increased globulin is not infrequently found years after all other abnormal elements have disappeared from the fluid.

In paralytica dementia, while much benefit may be expected in increasing the number and length of remissions, the ultimate hope for recovery is slight. When a paretic type of gold curve is found in the fluid of patients in whom the clinical diagnosis of paresis is not justified, the most intensive form of treatment should be instituted from the beginning. It is probable that the finding of this paretic type of gold curve often helps us to make a diagnosis of paresis before clinical symptoms of the disease are present. This early diagnosis with consequent early treatment may be of extreme importance in preventing the development of the outspoken condition. Finally, treatment must be individualized, given in courses, and the condition of the fluid determined at the end of each course and at the beginning of the subsequent course. In this way, the indication for kind of treatment, as well as the manner of response, is much more certainly determined than if we depend on clinical symptoms and objective findings alone.

THE MECHANISM OF UREA RETENTION IN NEPHRITIS.

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The present paper furnishes additional evidence in support of the view of the mechanism of urea retention in Bright's disease advanced by Widal and Javal in 1904 (1). Upon their conception of this mechanism was based the later work of Ambard and Weill (2) and of the author (3). On account of a present general misconception of the mechanism of urea retention, it seems advisable to add further evidence in favor of the hypothesis originated by the French school, although all credit for the conception must be given to these writers. It is not proposed at this time to discuss the respective merits of diagnostic tests of renal function, either in regard to their reliability or simplicity. These studies have been carried on with the view of establishing certain laws which have biological significance and are apparently valid.

For the purposes of the present paper the term urea retention is intended to mean a relatively increased concentration of urea in the blood and tissues, such as may occur over rather long periods of time in acute and chronic nephritis and is attributed to a disturbance in renal function.

It does not refer to the more rapid accumulation of urea which may occur as a result of partial or complete suppression of urine, due not to a specific defect in the function which has to do with the excretion of urea, but to a suspension of that function which has to do with the elimination of fluid through the kidneys. Such accumulation commonly occurs in oliguria due to passive congestion of the kidneys, or to any other cause. Neither does it refer to the long continued positive nitrogen balance which has been described by some authors.

Widal and Javal (1) kept a woman aged 34 years under observation for 4 months, during which time the nitrogen balance was carefully observed. The patient

had been ill for 1 year with chronic nephritis and the general condition remained unchanged during the period of observation. The urine albumin varied between 1.0 and 2.5 gm. per liter. The chloride balance was perfect. They found that the patient came into nitrogen equilibrium readily after changes in the nitrogen intake, though somewhat more slowly than a normal individual. They also found that the concentration of urea in the blood bore a direct relation to the diet, and on a constant nitrogen intake was practically constant. After changes in diet the blood urea could be restored to the former level by a return to the original diet. They succeeded in causing the concentration of urea in the blood to increase from 0.36 to 1.93 gm. per liter by increasing the nitrogen intake. As a result of their experiment, they concluded that the fluctuations in the level of blood urea were due to the changes in diet, and that the increased concentration of urea in the blood, which occurred in response to increased nitrogen intake, effected the elimination of increased amounts of urea through the kidneys. The organism therefore remained in nitrogen equilibrium. In other words, high nitrogen intake, relatively high blood urea, and high rate of excretion were found to be parallel and interdependent phenomena, as were low nitrogen intake, relatively low blood urea, and low nitrogen output. The term relative is used since, as has been repeatedly pointed out, there can be no absolute standard for the concentration of urea in the blood, either in health or in disease. The above hypothesis received further support from the work of Ambard and Weill (2), who demonstrated that the rate of urea excretion depends primarily on the concentration in the blood. Numerous authors have confirmed the observation of Widai and Javal on the dependence of the concentration of urea in the blood on the level of protein intake.

The present paper deals chiefly with the detailed study of two cases similar to the study made by Widai and Javal, with a simultaneous study of the state of the urea excretion function by the index of urea excretion, based on Ambard's laws, previously described (3). These two cases reveal the interrelationship between nitrogen intake, blood urea concentration, and nitrogen output, and show that changes in the separate factors may occur while no change in the actual ability of the kidneys to excrete urea is demonstrable. Additional data are presented, from other cases, which show in common a characteristic not previously described.

Methods of Observation.

The two patients first described were cases of chronic nephritis selected on account of their diminished urea-eliminating function, with little or no impairment of the function of water excretion. They

were ambulatory patients, apparently not in a progressing state of the disease, and were able to tolerate the dietary changes well. On account of the length of the experiments it was thought advisable to allow a somewhat more varied diet than is usual in metabolism experiments, but the protein-containing foods were analyzed frequently, and were not varied from day to day. Protein was given chiefly in the form of eggs, bread, and milk. The chloride and fluid intake were also kept constant. Urine and stools were collected for 24 hour periods and analyzed daily for nitrogen. Blood urea was determined about twice a week, with a simultaneous urine analysis on a carefully timed specimen, in order to compute the index of urea excretion.¹

Both the patients tolerated the regimen well and showed little or no change in the general condition at the end of the experiment.

Case 1.—C. P., male; age 48 years (Table I, Text-fig. 1).

Diagnosis.—General arteriosclerosis, chronic interstitial nephritis.

The patient was first admitted to the hospital on September 14, 1915, and was kept under observation until June 5, 1916. He complained of general weakness and loss of weight which had been progressing for 4 or 5 years. Physical examination revealed an advanced grade of peripheral arteriosclerosis. The heart was slightly enlarged. The rate and rhythm were normal. There were no murmurs. Fluoroscopic examination and the x-ray plate of the chest showed an enlarged aortic arch. The blood pressure was 180 systolic and 110 diastolic. The urine was clear, straw-colored, neutral; specific gravity 1.011. There was a heavy trace of albumin, but no sugar. Hyaline and finely granular casts were present, but not in large numbers. No red blood cells were found. The total elimination of phenolsulfonephthalein was 30 per cent in 2 hours on September 15.

The patient reentered the hospital in October and the nitrogen balance was determined from October 26 to December 29. The findings during this period

¹ The index of urea excretion is computed from the concentration of urea in the blood, the rate of urea excretion, the concentration of urea in the urine, and the weight of the patient, according to the following formula.

$$\text{Index of urea excretion (I)} = \frac{D \sqrt{C} \times 8.96}{\text{Wt.} \times \text{Ur.}^2}$$

D = gm. of urea excreted per 24 hours.

C = " " " per liter of urine.

Ur. = " " " " " blood.

Wt. = body weight of individual, in kilos.

For further details consult the description of the index in previous papers (3).

TABLE I.
Case I.

Date.	Weight.	Diet per 24 hrs.				Urine per 24 hrs.			Daily nitrogen balance.							Urea.		Blood pressure.	
		Protein.	Calories.	Sodium chloride.	Fluid.	Sp. gr.	Albumin per liter (Fabach).	Quantity.	Urea N per liter of urine.	Total N per liter of urine.	Total urine N in 24 hrs.	Stool N in 24 hrs.	Total N.			Urea per liter of blood.	Index of excretion (I).	Systolic.	Diastolic.
													Output in 24 hrs.	Intake in 24 hrs.	Balance.				
1915	kg.	gm.	gm.	gm.	cc.	gm.	cc.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	
Oct. 26	66.4	120.0	2,501	10.0	3,000	1,009	0.3	3,537	4.12	4.52	16.0	1.6	17.6	19.2	+1.6	0.825	18.4	190	120
" 27	65.2	120.0	2,501	10.0	3,000	1,010	0.2	2,719	4.25	5.30	14.4	2.0	16.4	19.2	+2.8	—	—	—	—
" 28	66.0	120.0	2,495	10.0	3,000	—	—	3,145	—	—	Lost.	3.0	—	19.2	—	—	—	—	—
" 29	65.2	120.0	2,495	10.0	3,000	1,010	0.25	2,540	5.18	6.25	15.9	3.2	19.1	19.2	+0.1	0.832	17.8	190	120
" 30	65.4	120.0	2,495	10.0	3,000	1,010	Tr.	3,265	4.32	5.04	16.5	2.5	19.0	19.2	+0.2	—	—	—	—
" 31	65.0	120.0	2,506	10.0	3,000	1,010	0.3	2,650	4.96	5.82	15.4	3.3	18.7	19.2	+0.5	—	—	—	—
Nov. 1	65.2	120.0	2,506	10.0	3,000	1,013	0.3	2,420	5.96	6.42	15.5	2.4	17.9	19.2	+1.3	—	—	—	—
" 2	65.4	120.0	2,506	10.0	3,000	1,012	0.3	2,930	4.45	5.15	15.1	2.7	17.8	19.2	+1.4	0.835	19.4	200	120
" 3	65.0	120.0	2,506	10.0	3,000	1,013	0.3	2,773	4.91	5.39	14.9	2.8	17.7	19.2	+1.5	—	—	—	—
" 4	64.8	120.0	2,506	10.0	3,000	1,010	0.25	2,568	4.81	5.79	14.9	3.1	18.0	19.2	+1.2	—	—	—	—
" 5	65.0	120.0	2,509	10.0	3,000	1,011	0.25	2,691	5.44	6.00	16.1	3.1	19.2	19.2	0	0.858	22.0	—	—
" 6	64.6	60.0	2,497	10.0	3,000	1,010	—	2,435	4.12	5.69	13.8	1.1	14.9	9.6	-5.3	—	—	197	110
" 7	64.6	60.0	2,497	10.0	3,000	1,010	0.25	1,931	4.64	5.52	10.7	3.5	14.2	9.6	-4.6	—	—	—	—
" 8	64.6	60.0	2,493	10.0	3,000	1,010	Tr.	2,553	2.99	3.78	9.7	1.0	10.7	9.6	-1.1	—	—	192	110
" 9	64.6	60.0	2,497	10.0	3,000	1,008	0.2	2,270	3.38	4.00	9.1	1.8	10.9	9.6	-1.3	0.569	17.4	200	130
" 10	64.6	60.0	2,497	10.0	3,000	1,007	0.1	2,073	3.16	3.88	8.0	1.1	9.1	9.6	+0.5	—	—	206	130
" 11	64.0	60.0	2,497	10.0	3,000	1,005	0.2	2,272	2.63	3.54	8.0	2.7	10.7	9.6	-1.1	—	—	—	—
" 12	64.8	60.0	2,493	10.0	3,000	1,008	0.2	2,026	3.41	3.91	7.9	1.6	9.5	9.6	+0.1	0.499	23.2	195	125
" 13	65.0	60.0	2,493	10.0	3,000	1,008	0.2	2,171	3.05	3.57	7.7	2.1	9.8	9.6	-0.2	—	—	190	110
" 14	64.8	60.0	2,493	10.0	3,000	1,009	0.25	1,988	3.42	3.82	7.6	1.7	9.3	9.6	+0.3	—	—	—	—

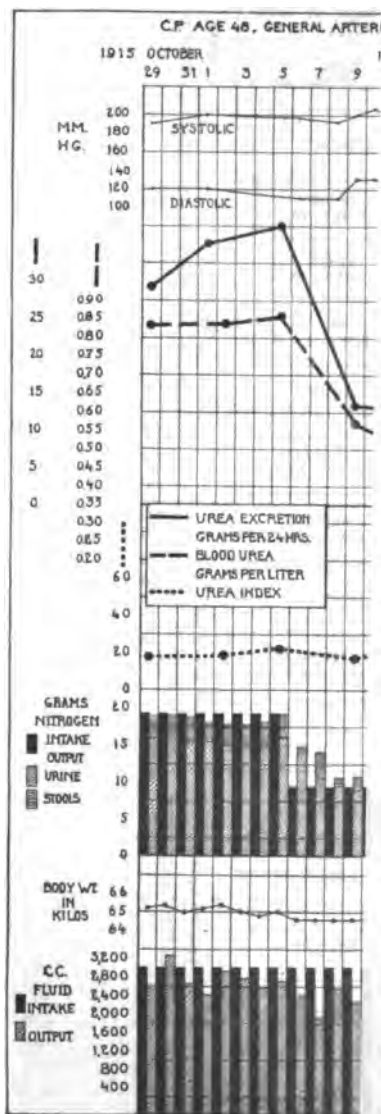
Nov. 15	64.6	60.0	2,497	10.0	3,000	1,007	0.1	1,918	2.75	3.50	6.7	1.5	8.2	9.6	+1.4	—	—	194	110
" 16	64.8	60.0	2,411	10.0	3,000	1,007	0.2	2,426	1.99	2.72	6.6	2.9	9.5	9.6	+0.1	0.411	24.0	—	—
" 17	64.8	60.0	2,411	10.0	3,000	1,006	0.25	2,251	2.66	3.19	7.2	1.9	9.1	9.6	+0.5	—	—	—	—
" 18	65.0	60.0	2,411	10.0	3,000	1,007	0.25	2,141	2.55	3.14	6.7	1.6	8.3	9.6	+1.3	—	—	—	—
" 19	65.4	60.0	2,411	10.0	3,000	—	Tr.	1,996	2.79	3.57	7.1	1.6	8.7	9.6	+0.9	0.402	21.0	—	—
" 20	65.0	30.0	2,798	10.0	3,000	1,006	0.2	1,915	2.26	3.15	6.0	1.1	7.1	4.8	-2.3	—	—	205	120
" 21	65.4	30.0	2,798	10.0	3,000	1,006	0.1	2,073	1.71	2.19	4.5	1.3	5.8	4.8	-1.0	—	—	—	—
" 22	65.0	30.0	2,798	10.0	3,000	1,006	Tr.	1,936	1.50	2.24	4.3	1.2	5.5	4.8	-0.7	—	—	—	—
" 23	64.6	30.0	2,798	10.0	3,000	1,005	0.2	2,177	1.36	1.78	3.9	1.2	5.1	4.8	-0.3	0.269	32.0	195	110
" 24	65.2	30.0	2,798	10.0	3,000	1,008	H.Tr.	1,852	1.27	1.97	3.6	1.6	5.2	4.8	-0.4	—	—	—	—
" 25	65.6	30.0	2,800	10.0	3,000	1,007	0.1	2,330	0.98	1.76	4.1	1.3	5.4	4.8	-0.6	—	—	—	—
" 26	65.4	30.0	2,798	10.0	3,000	1,005	Tr.	1,902	1.16	1.61	3.1	1.7	4.8	4.8	0	0.220	33.5	—	—
" 27	65.6	30.0	2,798	10.0	3,000	—	—	2,244	1.21	1.68	3.7	1.3	5.0	4.8	-0.2	—	—	210	130
" 28	65.4	30.0	2,798	10.0	3,000	—	Tr.	2,348	1.33	1.80	4.2	1.6	5.8	4.8	-1.0	—	—	—	—
" 29	65.0	30.0	2,793	10.0	3,000	1,007	0.3	1,873	1.37	2.00	3.7	1.5	5.2	4.8	-0.4	—	—	—	—
" 30	65.4	30.0	2,793	10.0	3,000	1,010	0.2	1,458	1.56	2.39	3.5	1.4	4.9	4.8	-0.1	0.211	21.4	—	—
Dec. 1	66.0	30.0	2,411	10.0	3,000	1,007	Tr.	1,896	1.72	2.49	4.7	1.3	6.0	9.6	+3.6	—	—	—	—
" 2	66.0	60.0	2,411	10.0	3,000	1,007	F.Tr.	2,249	1.62	2.29	5.2	2.1	7.3	9.6	+2.3	—	—	—	—
" 3	65.8	60.0	2,411	10.0	3,000	1,007	0.2	2,419	1.58	2.18	5.3	1.3	6.6	9.6	+3.0	0.272	19.1	—	—
" 4	65.8	60.0	2,411	10.0	3,000	1,008	—	2,319	1.84	2.40	5.6	2.3	7.9	9.6	+1.7	—	—	—	—
" 5	65.4	60.0	2,105	10.0	3,000	1,008	—	1,716	2.21	2.98	5.1	1.4	6.5	9.6	+3.1	—	—	—	—
" 6	65.8	60.0	1,995	10.0	3,000	1,009	0.2	1,598	2.56	3.32	5.3	1.4	6.7	9.6	+2.9	—	—	—	—
" 7	66.2	60.0	1,995	10.0	3,000	1,005	0.2	2,860	1.63	2.24	6.4	1.8	8.2	9.6	+1.4	0.363	19.2	200	120
" 8	65.4	60.0	1,995	10.0	3,000	1,010	0.4	1,637	2.72	3.59	5.9	1.1	7.0	9.6	+2.6	—	—	—	—
" 9	65.2	60.0	1,995	10.0	3,000	1,005	—	2,024	2.26	2.73	5.5	1.7	7.2	9.6	+2.4	—	—	180	120
" 10	65.8	60.0	1,995	10.0	3,000	1,006	—	2,412	1.93	2.52	6.0	2.0	8.0	9.6	+1.6	0.411	16.5	—	—
" 11	65.6	60.0	1,995	10.0	3,000	—	0.1	1,765	2.89	3.52	6.2	1.9	8.1	9.6	+1.5	—	—	—	—
" 12	65.6	60.0	1,995	10.0	3,000	—	0.1	2,710	2.15	2.42	6.5	1.8	8.3	9.6	+1.3	—	—	—	—
" 13	66.0	60.0	1,995	10.0	3,000	1,007	Tr.	2,051	2.37	3.08	6.3	1.2	7.5	9.6	+2.1	—	—	—	—
" 14	66.0	60.0	1,995	10.0	3,000	—	H.Tr.	2,292	2.32	2.81	6.45	1.9	8.35	9.6	+1.25	0.430	16.5	200	120

UREA RETENTION IN NEPHRITIS

TABLE I—*Concluded.*

Date.	Weight. kg.	Diet per 24 hrs.				Urine per 24 hrs.				Daily nitrogen balance.						Urea.		Blood pressure.	
		Protein. gm.	Calories.	Sodium chloride. gm.	Fluid. cc.	Sp. gr.	Albumin per liter (Babcock).	Quantity. cc.	Urea N per liter of urine.	Total N per liter of urine.	Total urine N in 24 hrs.	Stool N in 24 hrs.	Output in 24 hrs.	Intake in 24 hrs.	Balance.	Urea per liter of blood.	Index of excre- tion (I).	Systolic.	Diastolic.
1915																			
Dec. 15	66.0	60.0	1,995	10.0	3,000	1.008	—	2,145	2.31	2.86	6.2	2.2	8.4	9.6	+1.2	—	—	—	—
" 16	65.6	60.0	1,995	10.0	3,000	1.010	—	1,737	2.97	3.87	6.7	1.2	7.9	9.6	+1.7	—	—	—	—
" 17	65.8	59.9	1,998	10.0	3,000	1.007	—	2,381	2.16	2.89	6.9	2.0	8.9	9.6	+0.7	—	—	—	—
" 18	65.6	59.9	1,999	10.0	3,000	1.009	0.1	1,916	2.60	3.48	6.2	1.8	8.0	9.6	+1.6	—	—	—	120
" 19	65.4	59.9	1,989	10.0	3,000	1.007	0.1	1,886	2.49	3.30	6.2	2.8	9.0	9.6	+0.6	—	—	—	—
" 20	65.8	60.0	1,995	10.0	3,000	1.008	0.3	2,259	2.39	3.02	6.8	1.1	7.9	9.6	+1.7	—	—	—	200
" 21	65.6	60.0	1,995	10.0	3,000	1.007	0.3	2,219	2.18	2.78	6.2	2.0	8.2	9.6	+1.4	0.388	30.0	—	120
" 22	66.0	90.0	2,200	10.0	3,000	—	0.35	2,083	2.68	3.42	7.1	1.8	8.9	9.6	+0.7	—	—	—	—
" 23	65.8	90.0	2,200	10.0	3,000	1.006	—	2,016	2.44	3.06	6.2	2.4	8.6	9.6	+1.0	—	—	—	—
" 24	65.6	90.0	2,200	10.0	3,000	1.010	—	1,945	3.29	4.15	8.1	2.1	10.2	12.0	+1.8	0.506	15.0	—	—
" 25	—	90.0	2,190	10.0	3,000	1.009	0.4	2,146	3.26	3.99	8.6	1.7	10.3	12.0	+1.7	—	—	—	—
" 26	65.2	90.0	2,200	10.0	3,000	1.007	—	2,080	3.39	4.51	9.4	1.8	11.2	12.0	+0.8	—	—	—	—
" 27	65.8	90.0	2,200	10.0	3,000	1.010	0.1	2,469	3.13	3.66	9.0	2.6	11.6	12.0	+0.4	—	—	—	—
" 28	65.6	90.0	2,200	10.0	3,000	—	—	1,868	4.43	5.08	9.5	3.7	13.2	12.0	-1.2	—	—	—	—
" 29	65.2	2,200	10.0	3,000	—	—	0.3	1,897	4.27	5.10	9.6	0.6	10.2	12.0	+1.8	0.564	19.8	220	130





TEXT-

of observation are given in Table I. The patient tolerated the strict dietary regimen well, and complained only of gastric distress at times.

At the time of discharge from the hospital, the patient had gained in weight and strength, but there had been no change in the urinary or blood findings.

Case 2.—W. F., male; age 17 years (Table II, Text-fig. 2).

Diagnosis.—Chronic interstitial nephritis, aortic and mitral stenosis.

Admitted to the hospital on March 27, 1916. The patient seemed quite well, but was brought to the hospital on account of albuminuria. This had been discovered 1 year before, after a brother aged 18 years had died suddenly of uremia. The patient had had three attacks of rheumatism, 6, 4, and 2 years ago. Following the last attack, he had heart failure, with edema of both legs, which had been relieved by administration of a drug which caused a marked diuresis. No history of scarlet fever, measles, or diphtheria was obtained.

On physical examination the patient appeared to be a normal healthy boy aged 17 years, well nourished, and with good color. The heart was enlarged, and there was a systolic murmur at the apex and a diastolic murmur at the base. There were no signs or symptoms of heart failure. The blood pressure was 140 systolic and 85 diastolic. The urine was clear, straw-colored, and acid; specific gravity 1,012. There was a heavy trace of albumin, and no sugar. The sediment contained hyaline casts, leukocytes, and occasional red blood cells.

The patient was kept under close observation, and the nitrogen balance determined from March 28 to June 11. During this time there was no change in the general condition. On May 31, 40 gm. of urea were added to the diet, on June 1, 40 gm., and on June 2, 20 gm. No effect was noticed except that the patient asked for an increase in the daily allowance of fluid. The patient stated that he felt no change in his condition on or after these days.

Since discharge from the hospital the general condition has remained unchanged. When last examined on November 22, 1916, the urine was amber and turbid; specific gravity 1,012. 1.2 gm. of albumin per liter of urine were found (Esbach). There were a few casts in the sediment. The blood urea concentration was 0.774 gm. per liter and the urea index was 28.2.

Since Cases 1 and 2 are exactly similar, they will be discussed together. Both show the essential features described by Widai and Javal in their case; that is, a close parallelism between nitrogen intake, concentration of urea in the blood, and nitrogen output. Changes in nitrogen intake were always quickly followed by a change in the concentration of urea in the blood and in nitrogen output, so that nitrogen balance was reestablished on the new level. In Case 2 extremely wide fluctuations in the concentration of urea in the blood were obtained, from a minimum of 0.262 gm. per liter to a maximum of 2.542 gm. It is significant that the upper limit was far above the

TABLE II.
Case 2.

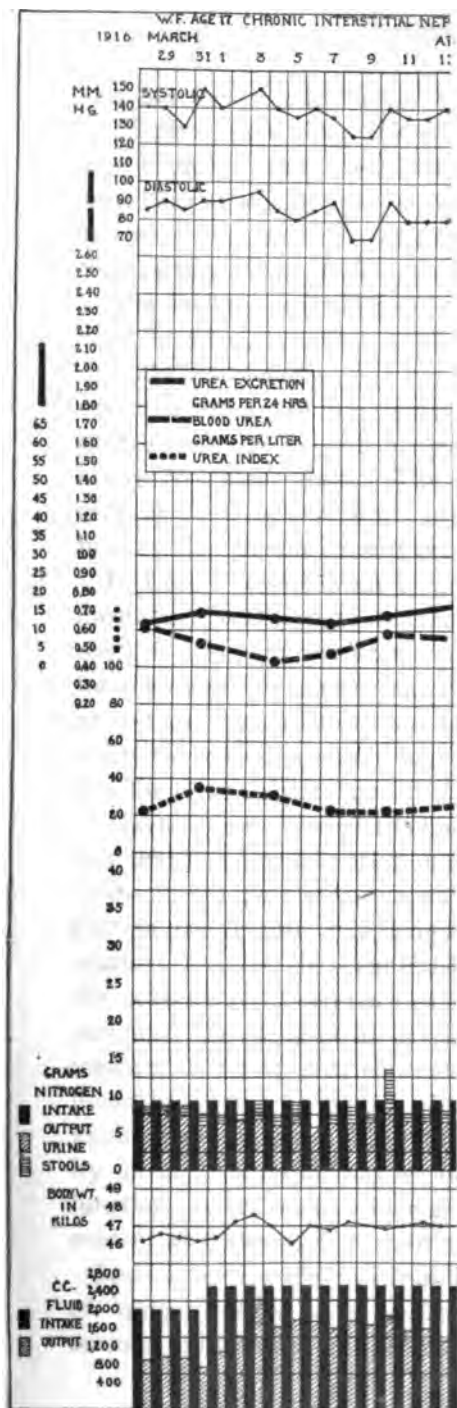
Date.	Weight.	Diet per 24 hrs.				Urine per 24 hrs.			Daily nitrogen balances.								Urea.		Blood pressure.	
		Protein.	Calories.	Sodium chloride.	Fluid.	Sp. gr.	Albumin per liter (Rabach).	Quantity.	Total N per liter of urine.	Total urine N in 24 hrs.	Stool N in 24 hrs.	Total N.			Urea per liter of blood.	Index of excretion (I).	Systolic.	Diastolic.		
												Output in 24 hrs.	Intake in 24 hrs.	Balance.						
1916		gm.		gm.	cc.		gm.	cc.	gm.	gm.	gm.	gm.	gm.	gm.	gm.					
Mar. 28	46.2	60.0	2,502	5.0	2,000	1.015	1.3	963	6.68	8.04	7.75	0.89	8.64	9.32	+0.68	0.610	22.5	140	85	
" 29	46.6	60.0	2,502	5.0	2,000	1.015	0.8	970	7.37	8.38	8.12	0.60	8.72	9.32	+0.60	—	—	140	90	
" 30	46.4	60.0	2,502	5.0	2,000	1.016	1.0	925	6.65	7.97	7.38	1.39	8.77	9.32	+0.55	—	—	130	85	
" 31	46.2	60.0	2,502	5.0	2,000	1.017	1.0	782	6.44	7.70	6.02	1.47	7.49	9.32	+1.83	0.524	35.0	150	90	
Apr. 1	46.4	60.0	2,502	5.0	2,500	1.015	0.5	1,095	4.75	5.70	6.25	0.87	7.12	9.32	+2.20	—	—	140	90	
" 2	47.2	60.0	2,502	5.0	2,500	1.011	0.2	1,415	4.34	4.74	6.71	0.13	6.84	9.32	+2.48	—	—	—	—	
" 3	47.6	60.0	2,502	5.0	2,500	1.007	0.4	2,196	2.56	3.09	6.78	2.42	9.20	9.32	+0.12	—	—	150	95	
" 4	47.0	60.0	2,502	5.0	2,500	1.009	0.3	1,589	3.21	3.78	6.01	1.14	7.15	9.32	+2.17	0.434	31.0	140	85	
" 5	46.0	60.0	2,502	5.0	2,500	1.009	0.3	1,751	2.96	3.62	6.34	2.32	8.66	9.32	+0.66	—	—	135	80	
" 6	47.0	60.0	2,502	5.0	2,500	1.009	0.5	1,731	2.69	3.33	5.76	—	5.76	9.32	+3.56	—	—	140	85	
" 7	46.8	60.0	2,502	5.0	2,500	1.010	0.2	1,557	3.45	4.05	6.30	0.86	7.16	9.32	+2.16	0.474	23.0	135	90	
" 8	47.2	60.0	2,502	5.0	2,500	1.009	0.3	1,750	3.13	3.65	6.39	2.36	8.75	9.32	+0.57	—	—	125	70	
" 9	47.0	60.0	2,502	5.0	2,500	1.009	0.3	1,656	3.36	3.89	6.45	0.62	7.07	9.32	+2.25	—	—	125	70	
" 10	46.8	60.0	2,500	5.0	2,500	1.011	0.4	1,831	3.92	4.60	8.41	5.04	13.45	9.32	-4.13	0.587	23.0	140	90	
" 11	47.0	60.0	2,500	5.0	2,500	1.010	0.2	1,505	3.74	4.40	6.66	0.98	7.64	9.32	+1.68	—	—	135	80	
" 12	47.2	60.0	2,500	5.0	2,500	1.010	0.3	1,565	3.65	4.24	6.65	1.49	8.14	9.32	+1.18	—	—	135	80	
" 13	47.0	60.0	2,500	5.0	2,500	1.012	0.25	1,300	3.98	5.02	6.53	1.50	8.03	9.32	+1.29	—	—	140	80	
" 14	47.0	60.0	2,500	5.0	2,500	1.011	0.5	1,141	4.11	5.06	5.78	0.78	6.56	9.32	+2.76	0.559	26.8	135	90	
" 15	47.4	60.0	2,500	5.0	2,500	1.013	0.35	1,050	4.48	5.64	5.93	2.24	8.17	9.32	+1.15	—	—	130	80	
" 16	47.0	60.0	2,500	5.0	2,500	1.011	0.2	1,068	4.52	5.65	6.03	1.76	7.79	9.32	+1.53	—	—	135	80	
" 17	47.0	60.0	2,500	5.0	2,500	1.012	0.4	1,331	3.82	4.91	6.54	1.91	8.45	9.32	+0.87	—	—	135	80	

Apr. 18	47.4	60.0	2,500	5.0	2,500	1,012	0.25	1,274	3.79	4.91	6.12	2.42	8.54	9.32	+0.78	0.562	29.0	135 80
" 19	47.6	60.0	2,503	5.0	2,500	1,011	0.7	1,066	3.23	4.16	4.44	1.46	5.90	4.53	-1.37	—	—	135 85
" 20	47.6	60.0	2,503	5.0	2,500	1,009	0.2	1,435	2.28	2.97	4.25	1.76	6.01	4.53	-1.48	—	—	135 85
" 21	47.8	60.0	2,503	5.0	2,500	1,008	0.2	1,537	2.04	2.63	4.04	1.10	5.14	4.53	-0.61	0.377	28.0	135 85
" 22	48.0	60.0	2,503	5.0	2,500	1,008	0.3	1,720	1.68	2.20	3.78	2.09	5.87	4.53	-1.34	—	—	145 90
" 23	47.2	60.0	2,503	5.0	2,500	1,007	0.2	1,375	1.69	2.27	3.12	1.71	4.83	4.53	-0.30	—	—	130 80
" 24	48.0	30.0	2,503	5.0	2,500	1,010	0.2	1,243	1.62	2.37	2.95	1.55	4.50	4.53	+0.03	0.288	26.0	140 90
" 25	47.8	30.0	2,503	5.0	2,500	1,007	0.5	1,355	1.61	2.25	3.05	0.37	3.42	4.53	+1.11	—	—	135 90
" 26	48.0	30.0	2,503	5.0	2,500	1,007	0.3	1,684	1.32	1.87	3.15	1.68	4.83	4.53	-0.30	—	—	150 100
" 27	48.0	30.0	2,503	5.0	2,500	1,006	0.2	1,220	1.66	2.26	2.76	2.16	4.92	4.53	-0.39	0.287	29.5	140 90
" 28	48.0	15.0	2,501	5.0	2,500	1,007	0.3	1,941	1.06	1.47	2.64	0.98	3.83	2.40	-1.43	—	—	140 85
" 29	48.0	15.0	2,501	5.0	2,500	1,008	0.35	1,225	1.52	2.16	2.64	0.69	3.33	2.40	-0.93	—	—	150 90
" 30	47.8	15.0	2,501	5.0	2,500	1,006	0.3	1,470	1.20	1.66	2.44	1.15	3.59	2.40	-1.19	—	—	145 85
May 1	47.8	15.0	2,501	5.0	2,500	1,007	0.25	2,056	1.04	1.42	2.92	0.63	3.55	2.40	-1.15	0.262	26.0	145 90
" 2	47.8	75.0	2,499	5.0	2,500	1,011	0.4	1,216	1.90	2.75	3.34	1.38	4.72	11.5	+6.78	—	—	145 85
" 3	47.6	75.0	2,499	5.0	2,500	1,011	0.5	1,146	2.68	3.43	3.93	2.48	6.41	11.5	+5.09	—	—	140 90
" 4	48.0	75.0	2,499	5.0	2,500	1,009	0.3	1,480	2.72	3.38	5.00	2.38	7.38	11.5	+4.12	0.485	21.0	150 90
" 5	48.0	75.0	2,499	5.0	2,500	1,008	0.2	1,717	2.77	3.31	5.68	2.19	7.87	11.5	+3.63	—	—	140 80
" 6	48.2	75.0	2,499	5.0	2,500	1,009	0.2	1,858	2.60	3.17	5.90	0.90	6.80	11.5	+4.70	—	—	135 90
" 7	48.0	75.0	2,499	5.0	2,500	1,009	0.3	1,540	3.23	4.00	6.15	1.10	7.25	11.5	+4.25	—	—	140 90
" 8	48.4	75.0	2,499	5.0	2,500	1,010	0.25	1,751	3.11	3.82	6.69	2.06	8.75	11.5	+2.75	0.656	13.3	132 85
" 9	48.8	75.0	2,499	5.0	2,500	1,010	0.4	2,286	2.34	2.99	6.85	0.71	7.56	11.5	+3.94	—	—	145 90
" 10	48.6	75.0	2,499	5.0	2,500	1,008	0.4	1,557	3.44	4.16	6.46	2.17	8.63	11.5	+2.87	—	—	140 95
" 11	48.8	75.0	2,499	5.0	2,500	1,007	0.5	1,686	3.66	4.47	7.54	2.01	9.55	11.5	+1.95	—	—	140 90
" 12	48.6	75.0	2,499	5.0	2,500	1,010	0.5	1,483	4.18	4.97	7.39	4.04	11.43	11.5	+0.07	0.603	25.5	—
" 13	48.6	75.0	2,499	5.0	2,500	1,010	0.45	1,634	4.29	4.98	8.15	1.91	10.06	11.5	+1.44	—	—	140 90
" 14	48.6	75.0	2,499	5.0	2,500	1,009	0.5	1,377	4.44	5.34	7.35	0.62	7.97	11.5	+3.53	—	—	—
" 15	48.6	75.0	2,499	5.0	2,500	1,008	0.5	1,378	4.26	5.16	7.11	1.48	8.59	11.5	+2.91	—	—	145 90
" 16	48.6	75.0	2,499	5.0	2,500	1,007	0.45	1,988	3.26	3.84	7.63	2.24	9.87	11.5	+1.63	0.611	22.5	135 80
" 17	48.6	120.0	1,782	5.0	2,500	1,008	0.45	1,498	4.08	4.81	7.20	2.56	9.76	18.4	+8.64	—	—	135 100
" 18	49.0	120.0	1,782	5.0	2,500	1,009	0.6	2,024	4.66	5.50	11.13	3.00	14.13	18.4	+4.27	—	—	145 80

TABLE II—Concluded.

Date.	Weight.	Diet per 24 hrs.				Urine per 24 hrs.			Daily nitrogen balance.							Urea.		Blood pressure.	
		Protein.	Calories.	Sodium chloride.	Fluid.	Bp. gr.	Albumin per liter (Ebach.)	Quantity.	Urea N per liter of urine.	Total N per liter of urine.	Total urine N in 24 hrs.	Blood N in 24 hrs.	Output in 24 hrs.	Intake in 24 hrs.	Balance.	Urea per liter of blood.	Index of excretion (I).	Systolic.	Diastolic.
1916	kg.	gm.	gm.	gm.	cc.	gm.	gm.	cc.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.		
May 19	49.2	120.0	1,782	5.0	2,500	1,010	0.5	1,921	5.24	6.08	11.70	2.56	14.26	18.4	+4.14	0.808	14.3	—	—
" 20	49.0	120.0	1,502	5.0	2,500	1,012	0.5	1,802	6.38	7.23	13.02	1.14	14.16	18.4	+4.24	—	—	130	80
" 21	49.0	120.0	1,502	5.0	2,500	1,010	0.5	1,826	6.65	7.39	13.50	1.82	15.32	18.4	+3.08	—	—	120	80
" 22	48.8	120.0	1,502	5.0	2,500	1,010	0.3	1,937	6.45	7.22	14.00	1.60	15.6	18.4	+2.8	—	—	140	90
" 23	48.2	120.0	1,502	5.0	2,500	1,011	0.3	1,775	6.92	7.62	13.52	3.66	17.18	18.4	+1.22	1.263	13.5	125	80
" 24	48.4	120.0	1,502	5.0	2,500	1,010	0.3	1,903	7.26	8.03	15.3	—	15.3	18.4	+3.10	—	—	140	90
" 25	49.0	120.0	1,502	5.0	2,500	1,011	0.5	1,778	7.75	8.44	15.0	1.68	16.68	18.4	+1.72	—	—	120	70
" 26	48.2	120.0	1,502	5.0	2,500	1,011	0.2	1,992	7.65	8.52	17.0	2.52	19.52	18.4	-1.12	1.314	18.1	140	90
" 27	48.0	120.0	1,502	5.0	2,500	1,014	0.2	1,930	8.00	8.66	16.70	1.95	18.65	18.4	-0.25	—	—	128	72
" 28	48.0	120.0	1,502	5.0	2,500	1,012	0.4	1,582	8.21	9.05	14.32	—	14.32	18.4	+4.08	—	—	—	—
" 29	47.6	120.0	1,502	5.0	2,500	1,010	0.3	2,429	6.46	7.04	17.92	0.73	17.75	18.4	+0.65	—	—	125	80
" 30	48.0	120.0	1,502	5.0	2,500	1,011	0.45	1,753	7.75	8.49	14.90	2.38	17.28	18.4	+1.12	1.192	18.8	125	80
" 31	48.0	120.0*	1,502	5.0	2,500	1,011	0.2	2,055	8.26	8.89	18.25	1.45	19.70	36.8	+17.1	—	—	120	75
June 1	47.8	120.0*	1,502	5.0	2,500	1,009	0.2	2,515	9.68	10.70	26.9	3.36	30.26	36.8	+6.54	—	—	125	80
" 2	47.0	120.0†	1,502	5.0	2,500	1,011	0.3	2,558	10.21	11.31	28.9	1.87	30.77	27.6	-3.17	2.542	8.3	120	75
" 3	46.6	60.0	2,502	5.0	2,800	1,010	0.3	1,548	9.65	10.40	16.1	1.32	17.42	9.32	-8.10	—	—	125	80
" 4	47.4	60.0	2,502	5.0	2,800	1,010	0.4	1,586	7.32	8.12	12.85	1.36	14.2	9.32	-4.88	—	—	—	—
" 5	48.0	60.0	2,502	5.0	2,800	1,008	0.5	1,812	4.27	5.18	9.40	2.04	11.44	9.32	-2.12	0.903	14.5	130	75
" 6	48.0	60.0	2,502	5.0	2,800	1,007	0.3	2,066	3.27	5.37	10.92	1.46	12.38	9.32	-3.06	—	—	130	75
" 7	48.2	60.0	2,502	5.0	2,800	1,006	0.3	1,647	2.66	4.75	7.82	4.3	12.12	9.32	-2.80	—	—	—	—
" 8	48.4	60.0	2,502	5.0	2,800	1,006	0.3	2,296	2.15	4.05	9.30	2.98	12.28	9.32	-2.96	0.470	22.2	130	85
" 9	48.6	60.0	2,502	5.0	2,800	1,007	0.2	2,142	2.34	4.07	8.72	1.44	10.16	9.32	-0.84	—	—	135	80
" 10	48.6	60.0	2,502	5.0	2,800	1,006	0.3	2,218	2.22	4.07	9.02	2.49	11.51	9.32	-2.19	—	—	130	80
" 11	48.6	60.0	2,502	5.0	2,800	1,006	0.2	2,135	2.18	4.08	8.72	1.79	10.51	9.32	-1.19	0.455	19.6	135	75

*40 gm. of urea added to diet.
†30 gm. of urea added to diet.



concentration in the blood at which Hewlett, Gilbert, and Wickett (4) believe that toxic effects of urea appear. The subject in this case is one in whom uremic symptoms should appear when the blood urea rises to a high level, if these symptoms are in any way referable to urea. As a matter of fact, no effect was noted beyond an increased fluid output, a slight loss in weight, and increased thirst.

The findings regarding urea excretion are of especial interest. In Case 1, with a variation in blood urea concentration from a minimum of 0.211 gm. per liter to a maximum of 0.858 gm., the index of urea excretion remained remarkably constant. In eighteen observations, with an average of 21.4, a figure below 16.4 is noted only once, and above 26.4 three times. The variations must be regarded as insignificant when the complexity of the formula and the possibility of error in obtaining the final result are taken into account.

In Case 2 a somewhat greater range of variation is noted. The most significant variation is that seen on June 2, when the blood urea reached 2.542 gm. per liter. Apparently the demand on the kidneys for the excretion of urea had exceeded their maximum capacity, and an actual accumulation had begun. This occurred, however, only when the nitrogen intake had been maintained at over 36 gm. per day for more than 2 days.

The two cases confirm the findings of Widai and Javal, and add the fact that no essential variation in the ability of the kidneys to respond to even high concentrations of urea in the blood at various levels of protein metabolism was demonstrable, until a very high level was reached; that is, the quantitative relationship that existed between the concentration of urea in the blood and the rate of its excretion remained the same at all levels of protein metabolism. The usual increase in blood urea concentration in nephritic individuals, on a liberal allowance of protein, can, therefore, hardly be attributed to diminished capacity for urea elimination. In these patients the capacity for urea excretion was far beyond the ordinary demands on that function, yet a relative increase in the blood urea concentration is evident at all levels of protein metabolism.

Several writers who have published observations obtained by means of Ambard's laws have emphasized the variations which occur in normal individuals. These variations, which may be quite wide,

are known to occur in the same individual and among different individuals. Attention has repeatedly been called to similar observations in previous papers (3). These variations, however, do not appear to affect the validity of Ambard's laws. Evidence that the laws are valid in as far as the variables considered are concerned seems conclusive. If this is true, then variations in the results must be accounted for by changes in the individual which introduce certain variable factors not represented in the formula. We have previously suggested that the variation may be due in part to such variable factors.

In the first place, it has been generally recognized that the results are more nearly constant in nephritic individuals than in normal individuals. The cases outlined above illustrate the constancy of the laws in two individuals with a marked disturbance in the elimination of urea. It is in normal individuals, as has already been noted, that a marked variability occurs in the index, although the findings do not, as a rule, give rise to doubt as to whether the individual falls within a normal or an abnormal group. Variability or elasticity of this sort probably represents a factor of safety. Its loss, therefore, would seem to be a sign of disease, even though this loss occurs before any quantitative diminution in renal function appears. If this assumption is correct, we should expect to find individuals in whom normal, or nearly normal quantitative estimations of renal function are found, but in whom, on repeated examination, there is the striking constancy in numerical results of the application of Ambard's laws that is shown by distinctly abnormal cases. These individuals would not, then, be considered normal. Cases 3 to 6 (Tables III to VI) show this constancy in numerical value of the index.

Case 3.—E. C., female; age 39 years (Table III).

Diagnosis.—Mitral stenosis, arterial hypertension, hypertrophy of heart.

The patient was under observation from December 2, 1915. She complained of headache, cough, and dyspnea on exertion, and presented the physical signs of mitral stenosis. There was no edema. Her blood pressure varied while under observation from 210 systolic and 130 diastolic, to 140 systolic and 80 diastolic after rest in bed. Examination of the urine on December 11 showed it to be clear, amber, acid, and to contain a very faint trace of albumin but no casts or

sugar; specific gravity 1.027. On repeated examination the amount of albumin varied from negative to a trace. Hyaline casts were found but once. The patient was subjected to alterations in diet, but the nitrogen balance was not determined. Her general condition improved with complete rest, but there was no change in the physical signs, except for lowering of blood pressure.

The total elimination of phenolsulfonephthalein on December 2 was 65 per cent in 2 hours.

The findings regarding urea excretion in the six observations which were made are tabulated in order, according to the concentration of urea found in the blood.

TABLE III.

Case 3.

Date.	Weight.	Urine in 24 hrs.	Urea.			Index of excretion (1%).
			Per liter of blood (Ur. °).	Per liter of urine (C°).	Amount in 24 hrs. (D°).	
1915-16	kg.	cc.	gm.	gm.	gm.	
Dec. 31.....	72.2	7,100	0.107	1.30	9.07	112
Jan. 26.....	69.6	9,300	0.170	1.69	15.7	91
Dec. 3.....	76.0	4,900	0.187	3.13	15.3	92
" 2.....	75.5	10,200	0.196	2.23	22.8	105
Jan. 11.....	72.0	2,020	0.252	8.82	17.85	104
Dec. 14.....	74.6	2,600	0.300	9.35	24.3	99

* For the explanation of these abbreviations see the formula for the index of urea excretion.¹

Case 4.—J. F., male; age 43 years (Table IV).

Diagnosis.—Mitral stenosis, auricular fibrillation, chronic heart failure.

The patient was admitted on November 27, 1915, and remained in the hospital until July 11, 1916, when he died. During the entire stay he presented a typical picture of chronic cardiac valvular disease with heart failure. Response to treatment was at times striking, but never prolonged, and the patient was never entirely free from edema. The amount of albumin in the urine varied from a very faint trace, at times when the elimination of urine was best, to 3.0 gm. per liter (Esbach) at times when the other signs of cardiac failure, such as dyspnea, cyanosis, edema, and scanty urine, were worst. Hyaline and granular casts were present in abundance at times, but were few in number or absent when the urine albumin was low in amount. Red blood cells of undetermined origin were constantly present. The findings regarding urea excretion also varied with the condition of the patient, quantitatively normal findings being present at times when other signs were favorable. The table shows four successive observations made during one of these periods. At this time the patient was voiding an amount

of fluid in excess of his intake, felt fairly well, and the edema was disappearing. The urine on March 11, was amber, turbid, and there was a very faint trace of albumin; specific gravity 1,019. The sediment contained red blood cells, but no casts were seen.

TABLE IV.

Case 4.

Date.	Weight.	Urine in 24 hrs.	Urea.			Index of excretion (I).
			Per liter of blood (Ur.).	Per liter of urine (C).	Amount in 24 hrs. (D).	
1916	kg.	cc.	gm.	gm.	gm.	
Mar. 7.....	63.0	1,220	0.345	18.15	22.1	112
" 8.....	62.6	900	0.294	17.65	15.9	108
" 9.....	62.0	1,200	0.279	13.4	16.1	110
" 12.....	60.8	1,280	0.333	17.2	22.0	121

Case 5.—M. G., male; age 58 years (Table V).

Diagnosis.—General arteriosclerosis, chronic myocarditis, and hypertrophy of heart.

The patient was admitted to the hospital on December 8, 1915, and remained under observation until April 3, 1916. On admission he complained of swelling of the feet and dyspnea on exertion. There was marked edema of both lower extremities. The heart was considerably enlarged, the rate was rapid, and the rhythm normal. A systolic murmur was heard at the apex. The urine was turbid, dark amber, acid, and contained 0.25 gm. of albumin per liter (Esbach); specific gravity 1,013. There was no sugar. The sediment contained many leukocytes and leukocytic and hyaline casts. After a few days in bed without change in condition a marked diuresis was induced with theocin (0.9 gm. daily for 3 days). The edema of the extremities rapidly disappeared, there being practically none demonstrable on December 17, although fluid in the chest persisted for some time longer. At this time there was only a faint trace of albumin in the urine, with numerous leukocytes, but no casts in the sediment. The general condition of the patient remained good. The table shows all observations made on urea excretion from the time of admission until January 5, 1916, when theocin was again administered. The patient later had an attack of hemiplegia, and one of bronchopneumonia, but was discharged from the hospital in fairly good condition on March 5, 1916. He died on May 31, of a second attack of hemiplegia.

TABLE V.

Case 5.

Date.	Weight.	Urine in 24 hrs.	Urea.			Index of excretion (I).	Medication.
			Per liter of blood (Ur.).	Per liter of urine (C).	Amount in 24 hrs. (D).		
<i>1915</i>	<i>kg.</i>	<i>cc.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>		
Dec. 9	91.6	1,060	0.403	19.43	20.6	55	Theocin. “ “
“ 12	91.6	2,000	0.374	13.8	27.6	72	
“ 13	91.0	1,535	0.381	14.1	21.7	55	
“ 14	89.2	2,840	0.336	7.78	22.1	55	
“ 15	86.4	5,360	0.232	6.4	34.2	167	
“ 16	83.8	3,400	0.234	6.98	23.7	123	
“ 20	82.2	2,160	0.384	14.1	30.4	85	
“ 23	80.2	2,360	0.361	12.1	28.6	85	
“ 27	78.0	1,840	0.461	19.6	36.0	86	
“ 30	78.0	1,620	0.440	20.7	36.6	89	
<i>1916</i>							
Jan. 3	78.6	2,000	0.457	19.85	39.7	97	
“ 5	77.8	1,800	0.416	17.4	31.3	87	

Case 6.—J. A., male; age 43 years (Table VI).

Diagnosis.—Chronic interstitial nephritis, hypertrophy of heart.

The patient was first admitted to the hospital in March, 1915, and has been under almost constant observation since that time. He suffers from frequent attacks of cardiac insufficiency associated with severe edema, ascites, and hydrothorax. He responds readily to treatment, and generally leaves the hospital in fairly good condition, without dyspnea, cyanosis, or edema. Table VI records the observations made during the patient's third stay in the hospital, and all the findings of the period from March 23 to April 20 are shown. An unusual constancy in the index of excretion is shown. During this time the patient was improving rapidly while taking digipuratum, 0.2 gm., and diuretin, 0.2 gm. daily, 5 days out of 7. The urine was clear, amber, and acid; specific gravity 1,009. There was a trace of albumin, and a few hyaline casts were seen in the sediment. Fluid excretion during the period was constantly in excess of the intake. At other times the findings have shown a marked difference from those detailed below. The patient is at present again in the hospital, having been admitted for the fifth time.

TABLE VI.

Case 6.

Date.	Weight.	Urine in 24 hrs.	Urea.			Index of excretion (I).
			Per liter of blood (Ur.).	Per liter of urine (C).	Amount in 24 hrs. (D).	
<i>1916</i>	<i>kg.</i>	<i>cc.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	
Mar. 23.....	85.6	1,745	0.326	9.53	16.62	50
" 24.....	84.8	1,300	0.312	11.6	15.1	56
" 25.....	84.0	2,600	0.310	7.46	19.4	59
" 27.....	78.6	1,400	0.310	9.4	13.15	48
" 28.....	77.2	940	0.300	12.22	11.5	52
" 30.....	76.6	860	0.337	14.52	12.5	51
Apr. 1.....	76.0	1,100	0.340	11.95	13.15	46
" 3.....	76.6	1,200	0.407	16.4	19.65	56
" 4.....	76.8	1,200	0.423	17.25	20.7	56
" 6.....	74.8	1,100	0.399	15.55	17.1	51
" 8.....	72.0	700	0.354	18.15	12.7	54
" 10.....	71.8	1,015	0.447	18.8	19.1	52
" 13.....	72.2	1,300	0.507	19.2	24.95	52
" 16.....	72.0	940	0.411	16.35	15.4	46
" 18.....	71.0	900	0.414	17.8	16.05	50
" 20.....	69.8	800	0.329	15.8	12.6	59

Cases 3, 4, and 5 show results regarding urea excretion resembling the normal as far as numerical value is concerned, but differing from healthy individuals in that they show no tendency to variation in the numerical value of the index. The significance of such findings has not yet been determined, but they have a distinct bearing on the physiology of urea excretion. We assume that as a result of some unknown pathological change, a fixation of certain factors involved in urea excretion which are variable in normal individuals has occurred. The numerical results remain constant, even when wide variations in the level of protein metabolism are brought about by changes in the diet. This was shown to be true in Case 3. Case 6 shows findings more definitely pathological, but here too is a high degree of constancy in the index.

In Case 5, during a time when the circulation was obviously poor and the urine showed evidence of renal passive congestion, the index was nearly constant at 55. The administration of theocin was followed by marked diuresis and a temporary increase in the index.

The edema disappeared as well as the signs of passive congestion. The index then remained constant at about 85. The index was accordingly capable of variation, but not in the manner that variation ordinarily occurs in normal individuals. One must conclude that the factors which are usually variable in normal individuals were unusually fixed in this individual. Case 4 resembled Case 5.

Case 3 was one of those we are accustomed to include in the group of primary hypertension, in view of the absence of demonstrable renal lesion. According to the view just presented, the kidneys of this patient have lost their normal elasticity. Whether this loss represents the forerunner of an actual impairment of renal function must be left for further investigation to determine.

DISCUSSION.

The experimental facts presented demonstrate that the occurrence of a relatively increased concentration of urea in the blood follows the increased resistance with which diseased kidneys oppose the passage of urea. When, as the result of feeding an increased amount of nitrogen, the concentration of urea in the blood rises, a parallel increase in the rate of excretion of urea occurs. When a point is reached at which the rate of urea excretion is kept equal to the rate of formation by the organism, the level of urea in the blood ceases to rise and the organism remains in nitrogen equilibrium. But when the nitrogen intake in this individual is diminished, urea is excreted for a time more rapidly than it is formed, until the level of urea in the blood falls and reaches a point such that the rate of urea excretion is again equal to the rate of urea formation, and the organism is once more in nitrogen equilibrium. The experiments illustrate the events occurring in the so called retention of urea.

Fluctuation in the level of urea in the blood thus occurs in abnormal individuals in exactly the same way in which it occurs in normal individuals. The mechanism of excretion remains the same, but on account of the increased resistance with which the kidneys oppose the passage of urea the rate of urea excretion becomes relatively less rapid than in the case of normal individuals, and the level of urea in the blood becomes relatively higher. It is the rate of urea excretion, relative to the other conditions found and relative to the usual nor-

mal rate, that the index of urea excretion, based on Ambard's laws, expresses in numerical form.

As has been shown, the index of urea excretion may vary within wide limits in normal persons. These limits may be known as the limits of normality, and their extremes usually lie between 80 and 200. Fluctuations to this extent must, we think, be emphasized as well as the degree of constancy on which insistence has until now been placed, and which originally supplied the evidence on which the laws of Ambard were based.

In certain individuals, however, not fluctuation but extreme constancy, to which we now apply the term fixation, is the rule. Fixation is, in some cases, too striking to be considered as due to coincidence. It is dwelt upon in this paper on account of the support which it gives to the laws of Ambard. It appears now to be desirable to distinguish between extreme constancy or fixation and the relative constancy which we find in normal persons. The relative constancy of normal individuals is intended to include, as has been stated, a certain fluctuation as far as numerical values are concerned. It must be taken to include that degree of fluctuation, almost always above the level of 80, which normal people often exhibit. Fluctuation of this type has been found by all observers and is greater than the range of experimental error. The cause and the significance of fluctuation is difficult to determine. It may depend either upon the influence of variable factors not determined as yet, and therefore not included in the formula, or on the fact that the ascertainable values now included in the formula do not hold that relation to each other which the laws imply. The latter interpretation we hold to be inadmissible because there is a sufficient degree of constancy, even under conditions where fluctuation occurs, to furnish strong evidence of the validity of the laws. In certain abnormal conditions where fixation has been shown to occur, the striking degree of constancy obtained must be interpreted as adding greatly to this evidence already furnished by the degree of constancy obtained in normal individuals. The pathological significance of this fixation, especially when it occurs within the limits of normality, is not yet determined.

Finally, we have failed to note uremic symptoms in a patient in whom the concentration of urea in the blood rose, under experimental conditions, to 2.542 gm. per liter.

CONCLUSIONS.

1. Urea retention, in the sense of a relatively increased concentration in the blood, is the result of increased resistance to the excretion of urea through the kidneys.

2. The relatively increased concentration of urea in the blood overcomes the increased resistance to excretion, and the organism is thereby maintained in nitrogenous equilibrium.

3. The laws formulated by Ambard for the excretion of urea apply in the condition of urea retention under a widely varying range of conditions, as to nitrogen intake and excretion.

4. The numerical value of Ambard's constant changes in urea retention, but the relation of the variable factors to one another remains otherwise unchanged.

5. In certain individuals, with otherwise normal findings in regard to urea excretion, an unusual degree of constancy, to which we have applied the term fixation, has been found in the numerical results obtained by the application of Ambard's laws. These individuals are regarded, as the result of this study, as probably abnormal, but the pathological significance of the fixation has not been determined.

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NEPHRITIS FROM THE STANDPOINT OF UREA EXCRETION.*

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That nephritis, and particularly the form commonly known as chronic interstitial nephritis, is associated with alterations in the excretion of nitrogenous waste products, especially urea, has long been known. During the past few years the introduction of simpler methods for quantitative determination of the nitrogenous constituents of the blood has led to numerous clinical studies of the condition now known as urea retention. In its proper sense this term would imply a continuous positive nitrogen balance, with a constantly increasing amount of urea in the blood and tissues. In actual use, however, the term is applied to any condition associated with an increased concentration of urea in the blood, without reference to the nitrogen balance, and the finding of a blood urea concentration higher than the average normal is called urea retention and is taken to indicate disturbed renal function.

It is my purpose to attempt to make clear what actually happens in the condition of urea retention. Urea retention in the sense in which the term is now used is not a continuous process of piling up urea in the body, due to an inability of the kidneys to excrete the large amounts of urea formed in the body. As I shall show, the kidneys may be actually able to excrete far greater amounts of urea than they are ordinarily called on to do, even in severe cases of nephritis, while the condition known as urea retention exists in these cases at the lower levels of protein metabolism just as it does at the higher, even though in the former instance the blood urea figure may be used within normal limits.

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The credit for the present conception of the mechanism of urea retention is due to Widal and Javal.¹ On their work were based the later studies of Ambard and Weill² and of myself.³

Widal and Javal kept a woman, aged 34, under observation for four months, during which time the nitrogen balance was carefully observed. The patient had been ill for one year with chronic nephritis. They found that she came into nitrogen equilibrium readily after changes in diet, though somewhat more slowly than a normal person. They also found that the concentration of urea in the blood bore a direct relation to the diet, and on a constant nitrogen intake was practically constant. After changes in diet, the blood urea could be restored to the former level by a return to the original diet. They succeeded in causing the concentration of urea in the blood to increase from 0.36 to 1.93 gm. per liter by increasing the nitrogen intake. As a result of their experiment, they concluded that the fluctuations in level of blood urea were due to the changes in diet, and that the increased concentration of urea in the blood, which occurred in response to increased nitrogen intake, effected the elimination of increased amounts of urea through the kidneys. In other words, high nitrogen intake, relatively high blood urea, and high rate of excretion were found to be parallel and interdependent phenomena, as were low nitrogen intake, relatively low blood urea, and low nitrogen output. The foregoing hypothesis received further support from the work of Ambard and Weill,² who demonstrated that the rate of urea excretion depends primarily on the concentration in the blood. Numerous observers have confirmed the observation of Widal and Javal on the dependence of the concentration of urea in the blood on the level of protein intake.

We have carried out studies of two cases similar to the study made by Widal and Javal, but in addition we have studied the state of the

1. Widal, F., and Javal, A.: *Compt. rend. Soc. de biol.*, 1904, **57**, 301, 303.

2. Ambard, L.: *Compt. rend. Soc. de biol.*, 1910, **49**, 411, 506. Ambard, L., and Weill, A.: *Jour. physiol. et path. gén.*, 1912, **14**, 753.

3. McLean, F. C., and Selling, L.: *Jour. Biol. Chem.*, 1914, **19**, 31. McLean, F. C.: *Jour. Exper. Med.*, 1915, **22**, 212, 366; *Clinical Determination of Renal Function by an Index of Urea Excretion*, *THE JOURNAL A. M. A.*, Feb. 5, 1916, p. 415.

urea excretion function, at various levels of protein metabolism, by the index of urea excretion previously described.³ The findings of Widal and Javal have been duplicated, and their conclusions confirmed, and we have shown that the state of the function of urea excretion remained unchanged during the experiments, though great changes in the concentration of urea in the blood were observed.

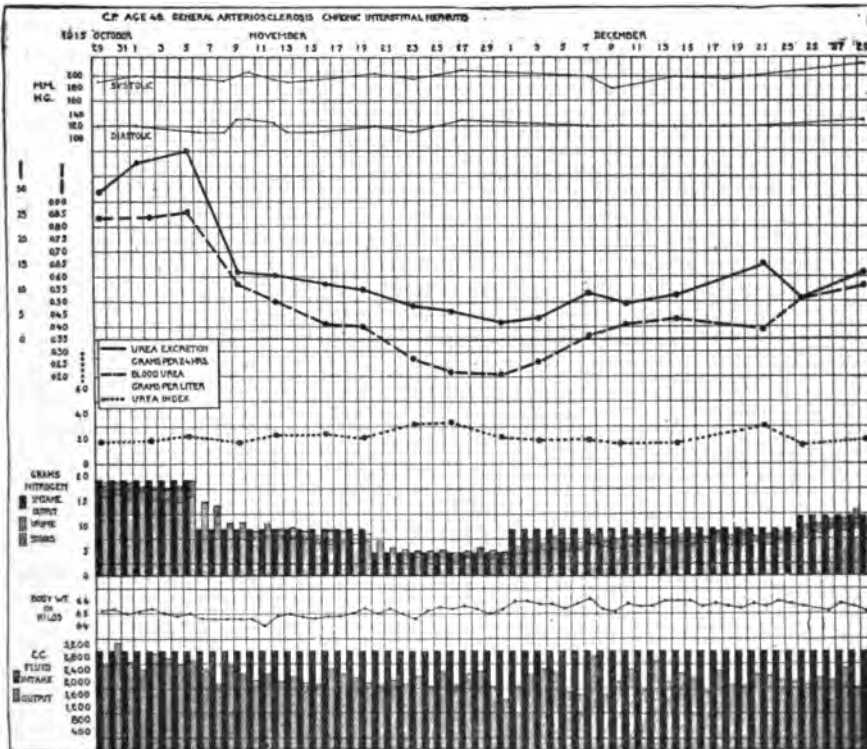


CHART 1. Findings in Case 1.

Methods of Observation.

The two patients studied had chronic nephritis, and were selected on account of an impairment of the function of urea excretion. Both were able to excrete large amounts of urine. They were both ambulatory patients, bore the strict dietary regimen well, and showed no change in general condition at the end of the experiment. Both are

still alive, more than a year after the conclusion of the experiments, and are in practically the same condition as at that time.

On account of the length of the experiments, the diet was varied more than is usual in metabolism experiments, but strict analysis was made of the protein-containing foods. The chlorid and fluid intake were kept constant. Urine and stools were collected in twenty-four hour periods, and analyzed daily for nitrogen. Blood urea was determined about twice a week, with a simultaneous urine analysis on a carefully timed specimen, in order to compute the index of urea excretion.⁴

The chief findings included in the two studied are given in the accompanying charts illustrating the two cases.⁵

Report of Cases.

Case 1.—C. P., man, aged 48, with general arteriosclerosis and chronic interstitial nephritis, first admitted to the hospital, Sept. 14, 1915, and kept under observation until June 5, 1916, complained of general weakness and loss of weight, which had been progressing for four or five years. There was found an advanced grade of general arteriosclerosis. The urine was clear, straw-colored and neutral; the specific gravity was 1.011. There was a heavy trace of albumin, but no sugar. Hyaline and finely granular casts were present, but not in large numbers. No red blood cells were found. The total elimination of phenolsulphonephthalein was 30 per cent. in two hours, September 15. Chart 1 shows the principal findings during a period of observation from October 26 to December 29.

4. The index of urea excretion is computed from the concentration of urea in the blood, the rate of urea excretion, the concentration of urea in the urine, and the weight of the patient, according to the following formula:

$$\text{Index of Urea Excretion (I)} = \frac{8.96 \times D \sqrt{C}}{\text{Wt.} \times \text{Ur}^2}$$

D, gm. of urea excreted per 24 hours;

C, gm. of urea per liter of urine;

Ur, gm. of urea per liter of blood;

Wt, body weight of individual, in kilograms. For further details consult the description of the index in previous papers.

5. Complete data in regard to the two cases here illustrated are given in Jour. Exper. Med., 1917, 26, 181.

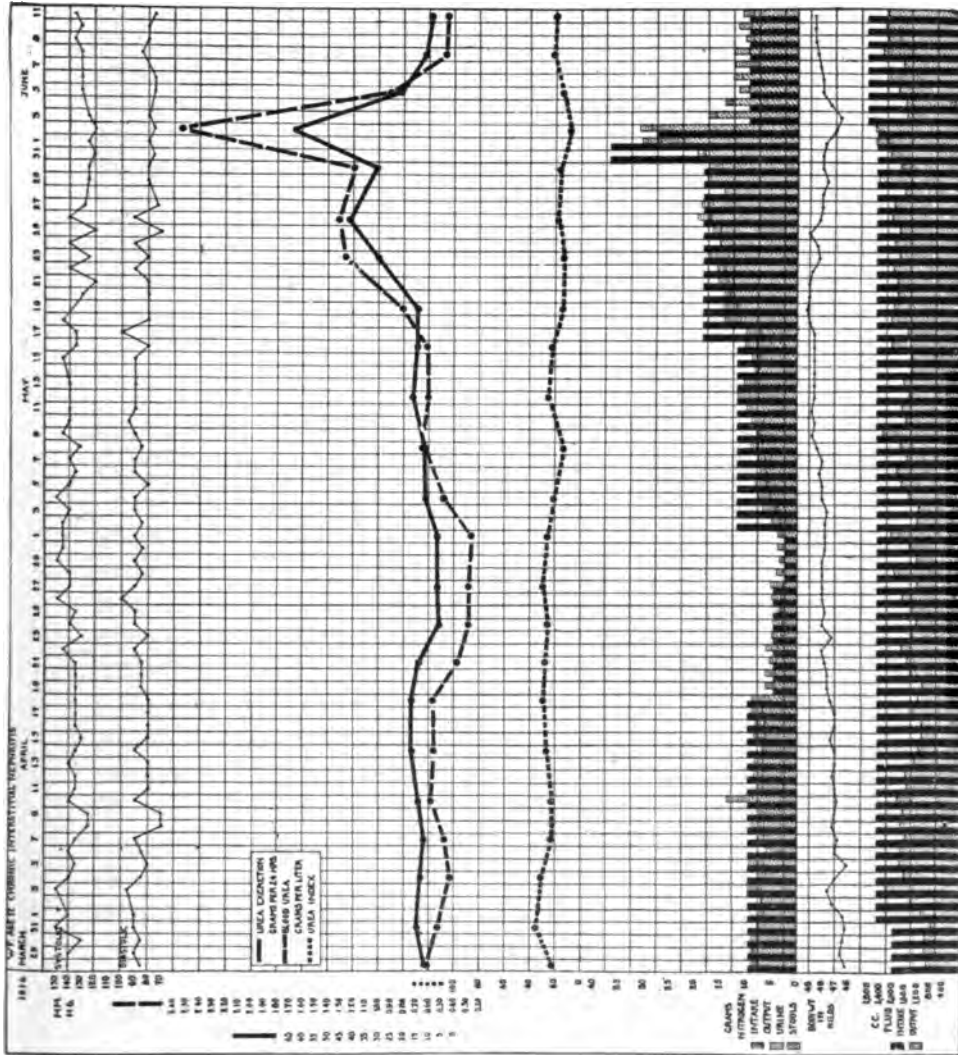


CHART 2. Findings in Case 2.

Case 2.—W. F., boy aged 17 years, with chronic interstitial nephritis and aortic and mitral insufficiency, admitted to the hospital, March 27, 1916, seemed quite well, but was brought to the hospital on account of albuminuria. This had been discovered about one year before, after a brother aged 18 years had died suddenly of uremia. The patient had had three attacks of rheumatism, six, four and two years before. Following the last attack he had heart failure, with edema of both legs. No history of scarlet fever, measles or diphtheria was obtained. On examination there were found the physical signs of mitral insufficiency, but there were no signs or symptoms of heart failure. The urine was clear, straw-colored and acid; the specific gravity was 1.012. There was a heavy trace of albumin and no sugar. The sediment contained hyaline casts, leukocytes, and occasional red blood cells. Chart 2 shows the principal findings during a period of observation from March 28 to June 11.

Comment.

The two cases are exactly similar. Both show the essential features described by Widal and Javal in their case; that is, a close parallelism between nitrogen intake, concentration of urea in the blood, and nitrogen output. A change in nitrogen intake was always followed quickly by a change in the concentration of urea in the blood and in nitrogen output, so that nitrogen balance was reestablished on the new level. In Case 2 there were produced extremely wide fluctuations in the concentration of urea in the blood, from a minimum of 0.262 gm. per liter to a maximum of 2.542. This maximum is far above the level at which Hewlett, Gilbert and Wickett⁶ believe that toxic effects of urea appear. It would seem that this patient, having suffered for some time with chronic nephritis, should have shown uremic symptoms, if these symptoms are in any way referable to an increased amount of urea in the blood and tissues. As a matter of fact, no effect was noted beyond an increased fluid output, a slight loss in weight, and increased thirst.

The findings regarding urea excretion are of especial interest. In Case 1, with a variation in blood urea concentration from a minimum

6. Hewlett, A. W., Gilbert, Q. O., and Wickett, A. D.: The Toxic Effects of Urea on Normal Individuals, *Arch. Int. Med.*, November, 1916, p. 636.

of 0.211 gm. per liter to a maximum of 0.855 gm., the index of urea excretion remained remarkably constant. In eighteen observations, with an average of 21.4, a figure below 16.4 is noted only once, and above 26.4 only three times. These variations must be regarded as insignificant when the complexity of the formula and the possibility of error in the various determinations are taken into account. In Case 2 there is a somewhat greater range of variation. The most significant variation is that seen, June 2, when the blood urea reached 2.542 gm. per liter. On this day the urea index was 8.3 instead of about 20. It would seem that here the maximum capacity of the kidneys to excrete urea had been exceeded and actual accumulation had begun. This occurred, however, only when the nitrogen intake had been maintained at over 36 gm. per day for two days.

SUMMARY.

The two cases confirm the findings of Widal and Javal, and add the fact that no essential variation in the ability of the kidneys to respond to even high concentration of urea in the blood at various levels of protein metabolism was demonstrable, until a very high level was reached; that is, the quantitative relationship that existed between the concentration of urea in the blood and the rate of its excretion remained the same at all levels of protein metabolism.

It has been demonstrated, therefore, that a relatively increased concentration of urea in the blood follows the increased resistance with which diseased kidneys oppose the passage of urea. When, as the result of feeding an increased amount of nitrogen, the concentration of urea in the blood rises, a parallel increase in the rate of excretion of urea occurs. When a point is reached at which the rate of urea excretion is kept equal to the rate of formation by the organism, the level of urea in the blood ceases to rise, and the organism remains in nitrogen equilibrium. But when the nitrogen intake in the individual is diminished, urea is excreted for a time more rapidly than it is formed, until the level of urea in the blood falls, and reaches a point such that the rate of urea excretion is again equal to the rate of urea formation, and the organism is once more in nitrogen equilibrium. This experiment illustrates the events occurring in the so-called retention of urea.

CONCLUSIONS.

1. Urea retention in the sense of a relatively increased concentration in the blood is the result of increased resistance to the excretion of urea through the kidneys.

2. The relatively increased concentration of urea in the blood overcomes the increased resistance to excretion, and the organism is thereby maintained in nitrogenous equilibrium.

3. The laws formulated by Ambard in regard to urea excretion apply in the condition of urea retention under a widely varying range of conditions, as to nitrogen intake and excretion.

4. The numerical value of Ambard's constant changes in urea retention, but the relation of the variable factors to one another remains otherwise unchanged.

5. The occurrence of a high concentration of urea in the blood is not necessarily accompanied by any symptoms suggestive of uremia.

VARIATIONS IN INFANTS OF TOTAL BLOOD SOLIDS AND THE CONCENTRATION OF SODIUM CHLORID IN THE PLASMA.

BY ANGELIA M. COURTNEY AND HELEN L. FALES.

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Babies' Hospital.)*

In the course of some investigations at the Babies' Hospital on nutritional edema of infants, determinations of total solids of the blood and chlorid content of the plasma were made on a large number of children. Some interesting facts which were brought out in the course of this work are here presented. A discussion of the significance of the findings will not be attempted. Therefore, a review of the extensive literature on the osmotic pressure of the blood and the relation of blood water and salts to the fluids in the tissues, conditions which have been supposed to have some importance in the production of this form of edema, is hardly called for here.

The literature of the two subjects which are considered in this communication is very limited. We have been unable to find reported any results of chlorid determinations in the plasma of infants' blood. Also, very few figures are obtainable on total solids of the blood, although the subject has been discussed by many pediatricists. Lederer¹ determined the blood water of suckling dogs. Lust² has reported a number of observations on blood water of normal infants. His results will be referred to later. Gettler and Baker,³ at Bellevue Hospital, have carried out a very complete investigation of the blood of normal adults. The results given for total solids and chlorid in the plasma of adults are of especial interest for comparison with the figures obtained by us for normal infants.

1. Lederer: *Ztschr. f. Kinderh.*, 1914, 10, 367.
2. Lust: *Jahrb. f. Kinderh.*, 1911, 73, 85 and 179.
3. Gettler and Baker: *Jour. Biol. Chem.*, 1916, 25, 211.

In all, seventy-nine determinations of blood solids and eighty-one of blood chlorid were made on sixty-seven different children. The ages ranged from 5 weeks to 6 years, but most of the subjects were under 2 years. Thirteen were well-nourished normal children from 2 months to 6 years of age; some of them were breast fed, some were fed with modifications of cow's milk and some were on a mixed diet. The remainder were suffering from quite a wide variety of diseases, the principal groups being lobar pneumonia, bronchopneumonia, tetany, marasmus and intestinal disturbance, nutritional edema, sclerema, and one case of poliomyelitis. Three of the children had highly concentrated blood. The ages of the abnormal children corresponded closely with those of the normal children. The diet was various with these also.

Technic Employed.—About 5 c.c. of blood were drawn into a syringe. One portion was transferred to a platinum dish and the remainder to a tube containing about a gram of powdered potassium citrate. The portion in the platinum dish was used to determine the total solids. This was immediately weighed, evaporated on the waterbath, and dried to constant weight in an oven at about 100°C. Total solids are reported as per cent. by weight. The citrated blood was centrifugalized to separate the plasma from the cells. The protein was then removed from the plasma according to the method of McLean and Van Slyke for the determination of blood chlorid.⁴ In the beginning their method was used for the determination of the chlorid in the filtrate. As identical results were obtained by the simpler Volhard method, however, most of the determinations of chlorid, after the preliminary removal of the protein, were made by the Volhard method. The figures are reported as grams of sodium chlorid in 1 c.c. plasma.

Normal Infants.—The results obtained with normal infants are shown in Table 1. Comparing the averages here with those of Gettler and Baker for adults (Table 9), it is seen that the values for chlorid are almost identical, while those for blood solids show quite a marked difference, the range in infants being from 16 to 18, in adults from 20 to 25 per cent. of the total blood. Lust reports an average of 18 per cent. blood solids for normal infants, which agrees fairly well with our own findings. The range of values found for normal infants is narrower than that reported for adults, in respect both to blood solids and chlorid. A study of these findings does not reveal any corre-

4. McLean and Van Slyke: Jour. Biol. Chem., 1915, 21, 361.

TABLE 1.
Normal Children.

Name	Date	Age, Months	Water in Blood, Per Cent. by Weight	Total Solids, Per Cent. by Weight	Chlorid in 1 C.c. Plasma Computed as Gm. of NaCl
Tessie R.....	Oct. 6	54	82.39	17.61	0.00577
Julius H.....	Oct. 10	48	82.43	17.57	0.00592
Helen H.....	Oct. 10	72	81.83	18.17	0.00563
Lawrence S.....	Oct. 23	20	82.23	17.77
Emmanuel D.....	Oct. 23	14	82.35	17.65
John D.....	Oct. 23	2	0.00626
Ralph S.....	Oct. 23	5	83.69	16.31	0.00574
Ernest A.....	Oct. 24	6	82.95	17.05	0.00641
Paul S.....	Oct. 24	17	83.77	16.23	0.00577
Dorothy C.....	Oct. 26	7	82.19	17.81	0.00595
Frank M.....	Oct. 31	8	81.73	18.27	0.00600
Henry R.....	Nov. 4	14	82.41	17.59	0.00536
John M.....	Nov. 8	17	83.30	16.70	0.00577
Average.....			82.61	17.39	0.00587

spondence between the variations in blood solid content and those in sodium chlorid in the plasma. The extreme values in each correspond to average values in the other.

Determinations were made on the blood of a girl of 4 years 8 months, convalescent from infantile paralysis. These values are not included in any of the averages (Table 9). The sodium chlorid value is normal, that for blood solids much higher than the average for children.

Pneumonia.—Table 2 gives the results in four children with bronchopneumonia and in five children with lobar pneumonia, the ages ranging from 10 to 30 months. The averages in the two groups are almost identical, both for solids and blood chlorid. But little importance is to be attached to the average for blood solids in either group, because of the wide variation met with. The average for blood chlorid is distinctly below normal; but there are two values in each group which are nearly normal. In the pneumonia cases as well as in the group of normal children there seems to be no correspondence between the variations in the blood solids and those in the sodium chlorid of the plasma.

TABLE 2.

Pneumonia.
Bronchopneumonia.

Name	Date	Age, Months	Water in Blood, Per Cent. by Weight	Total Solids, Per Cent. by Weight	Chlorid in 1 C.c. Plasma Computed as Gm. of NaCl
Andrew N.....	Oct. 10	15	82.05	17.95	0.00500
Joseph W.....	Oct. 10	20	85.81	14.19	0.00534
Josephine C.....	Oct. 13	10	82.90	17.10	0.00545
Carl H.....	Dec. 21	12	82.43	17.57	0.00456
Average.....			83.30	16.70	0.00509

Lobar Pneumonia.

Thomas W.....	Oct. 10	30	83.71	16.29	0.00538
Gertrude G.....	Oct. 10	33	83.66	16.34	0.00432
Rosemary D.....	Oct. 10	10	82.62	17.38	0.00485
Isabella S.....	Oct. 10	12	85.02	14.98	0.00473
Thomas L.....	Dec. 20	15	81.99	18.01	0.00583
Average.....			83.40	16.60	0.00502

Tetany.—The blood of ten children suffering from tetany of mild or severe type was examined. The ages ranged from 7 to 17 months. The results are shown in Table 3. The first four cases reported were

TABLE 3.

Tetany.

Name	Date	Age, Month	Water in Blood, Per Cent. by Weight	Total Solids, Per Cent. by Weight	Chlorid in 1 C.c. Plasma Computed as Gm. of NaCl
Ferdinand E.....	Dec. 21	17	82.39	17.61	0.00468
Edwin C.....	Jan. 20	12	82.61	17.39	0.00549
Peter G.....	Jan. 23	12	81.35	18.65	0.00482
Marcus C.....	Mar. 28	14	0.00533
Edith McM.....	Mar. 19	15	81.51	18.49	0.00458
Edith McM.....	Mar. 22	15	79.34	20.66	0.00503
Rose G.....	Dec. 1	17	83.18	16.82	0.00510
George I.....	Dec. 1	9	84.67	15.33	0.00582
Billie C.....	Dec. 19	13	81.28	18.72	0.00494
Mary S.....	Jan. 12	7	82.52	17.48	0.00501
Gladstone F.....	Mar. 28	14	0.00545
Average.....			82.09	17.91	0.00512

children with mild or moderate types of tetany; the remaining six all showed well-marked symptoms. The values for blood solids generally are higher than in normal children. The chlorid average is much lower than the normal, with only four values which even approximate it. No regularity can be seen in the relation of blood solids to the chlorid in the plasma in children with tetany.

Marasmus.—Table 4 shows the results of examination of the blood of twenty-three children suffering either from marasmus or from pro-

TABLE 4.
Marasmus and Intestinal Disturbance.

Name	Date	Age, Months	Water in Blood, Per Cent. by Weight	Total Solids, Per Cent. by Weight	Chlorid in 1 C.c. Plasma Computed as Gm. of NaCl
Mary McG.....	Sept. 30	6	83.26	16.74	0.00370
Mary McG.....	Oct. 13	6	83.44	16.56	0.00540
Florence H.....	Oct. 4	9	82.56	17.44	0.00519
Solomon B.....	Oct. 4	12	83.02	16.98
Agnes L.....	Oct. 4	6	82.96	17.04	0.00500
Salvatore L.....	Oct. 4	5*	83.77	16.23	0.00523
Martin C.....	Oct. 4	8	81.88	18.12	0.00454
John L.....	Oct. 4	9	82.73	17.27	0.00442
Walter K.....	Oct. 4	6	83.50	16.50	0.00468
Thaddeus E.....	Oct. 4	6	83.63	16.37	0.00503
Edith F.....	Oct. 6	6	83.07	16.93	0.00447
George N.....	Oct. 6	3	83.90	16.10	0.00508
Leonie W.....	Oct. 10	8	83.10	16.90	0.00412
Henrietta F.....	Oct. 10	8	82.12	17.88	0.00500
Alfred D.....	Oct. 10	7	81.37	18.63	0.00476
Alfred D.....	Oct. 26	8	82.11	17.89	0.00576
August S.....	Oct. 13	6	82.50	17.50	0.00478
James T.....	Oct. 13	48	83.62	16.38	0.00396
Grace D.....	Oct. 13	8	83.90	16.10	0.00515
Anna E.....	Oct. 13	8	83.45	16.55	0.00500
Mary T.....	Sept. 30	9	82.25	17.75	0.00413
Mary T.....	Oct. 6	9	83.15	16.85	0.00353
John A.....	Oct. 6	6	81.40	18.60	0.00461
John A.....	Oct. 13	6	83.86	16.14	0.00412
Albert H.....	Oct. 24	12	81.21	18.79	0.00483
Minnie C.....	Jan. 13	16	83.63	16.37	0.00545
Thomas S.....	Jan. 25	3	83.90	16.10	0.00560
Average.....			83.94	17.06	0.00475

* Weeks.

longed intestinal disturbance. The ages ranged from 5 weeks to 4 years, but most of the children were under 1 year. The average for blood solids is slightly below the normal average; that for blood chlorid very much below the normal. The range of values for blood solids, however, is very similar to that found in normal children. In this group, also, no regularity can be seen in the correspondence of blood solids and blood chlorid.

Concentrated Blood.—Three children among those examined showed abnormally concentrated blood. All of them had previously suffered from gastro-enteritis. In one, the blood concentration appeared to be the result of a transfusion; another had suffered from a severe

TABLE 5.
Concentrated Blood.

Name	Date	Age, Month	Water in Blood, Per Cent. by Weight	Total Solids, Per Cent. by Weight	Chlorid in 1 C.c. Plasma Computed as Gm. of NaCl	Hemo- globin, Per Cent.
Bernark K.....	Oct. 6	8	80.34	19.66	0.00318	...
William C.....	Oct. 4	1	77.12	22.88	0.00541	140
William C.....	Oct. 13	1½	76.25	23.75	0.00412	140
Richard D.....	Oct. 4	5½	80.37	19.63	140
Richard D.....	Oct. 13	6	79.20	20.80	0.00536	
Average.....			78.66	21.34		

burn; a third was deeply cyanosed at the time the blood was taken. The first and second cases had very high hemoglobin, in both instances reaching 140 per cent. The findings in these children are reported as a separate group in Table 5. Two examinations were made of the blood of each of the children with high hemoglobin. The five values given for blood solids show a wide range, but are all in a totally different class from those of all the other children studied. These values approximate the values for normal adults. The chlorid values vary so greatly that an average of the four would have little significance. It is worth noting that one extremely low value is found in a child who had received, on the preceding day, a large subcutaneous injection of bicarbonate of soda and more of the same drug by mouth.

Nutritional Edema.—In Table 6 are given the results of eleven determinations of blood solids and blood chlorid in five children suffering from nutritional edema. The ages ranged from 2 months to 2 years. The findings in this group show a strikingly low average for blood solids. In not a single instance is the value as high as the average given for normal children, and in only one is the value as high as the lowest in the normal group. The values for blood chlorid do not differ greatly from the values found in the other pathologic groups,

TABLE 6.
Nutritional Edema.

Name	Date	Age, Months	Water in Blood, Per Cent. by Weight	Total Solids, Per Cent. by Weight	Chlorid in 1 Cc. Plasma Computed as Gm. of NaCl
Seraphine C.....	Sept. 30	24	86.28	13.72	0.00437
Seraphine C.....	Oct. 13	..	84.93	15.07	0.00511
Seraphine C.....	Nov. 14	..	85.51	14.49	0.00590
Mathilda F.....	Sept. 26	15	85.21	14.79	0.00417
Mathilda F.....	Oct. 4	..	83.53	16.47	0.00511
Mathilda F.....	Oct. 13	...	84.70	15.30	0.00511
Mary F.....	Oct. 10	3	87.30	12.70	0.00468
Mary F.....	Oct. 14	..	87.09	12.91	0.00550
Louise S.....	Oct. 30	4	84.92	15.08	0.00536
Louise S.....	Nov. 6	0.00445
Marie K.....	Jan. 20	31	87.29	12.71	0.00510
Average.....			85.68	14.32	0.00490

the average being much below normal. The range, however, is wide, including several approximately normal figures.

Sclerema.—Three children, aged 2 months, 4 months and 2 months, respectively, having sclerema, were examined. The results are given in Table 7. The average for blood solids in this group is somewhat below normal, but does not at all resemble that obtained in the group of nutritional edema. Blood chlorid in two of the cases is normal, in the third it is extremely low. An injection of bicarbonate of soda had been given to this child on the previous day.

After Bicarbonate Injection.—During the investigation three children in all had received subcutaneously a bicarbonate of soda injection

TABLE 7.

Sclerema.

Name	Date	Age, Months	Water in Blood, Per Cent. by Weight	Total Solids, Per Cent. by Weight	Chlorid in 1 C.c. Plasma Computed as Gm. of NaCl
James V.....	Oct. 14	2	83.73	16.27	0.00274
George K.....	Oct. 28	2	83.48	16.52	0.00575
Nathaniel D.....	Oct. 31	4	83.23	16.77	0.00644
Average.....			83.48	16.52	0.00498

within the twenty-four hours preceding the blood examination. The findings are grouped together in Table 8. The sodium bicarbonate injection may possibly have been the cause of the extremely low values here seen for chlorid in the plasma, but, on the other hand, several other children, in the marasmus and intestinal disturbance group, who had not received such an injection show similarly low values. In this connection a recent communication by K. Goto,⁵ on blood studies in

TABLE 8.

After Injection of Sodium Bicarbonate.

Name	Date	Age, Months	Injection	Water in Blood, Per Cent. by Weight	Total Solids, Per Cent. by Weight	Chlorid in 1 C.c. Plasma Computed as Gm. of NaCl
John A.....	Oct. 13	6	Oct. 11—120 c.c.	83.86	16.14	0.00412
Bernard K.....	Oct. 6	8	Oct. 5—240 c.c.	80.34	19.66	0.00318
James V.....	Oct. 14	2	Oct. 13—120 c.c.	83.73	16.27	0.00274

uranium nephritis in the dog and the protective action of sodium bicarbonate, is of interest. He finds that the increase of chlorid in the plasma observed in uranium nephritis is not so great when sodium bicarbonate is administered at the time.

After Saline Injection.—In ten children a physiologic salt solution had been injected subcutaneously shortly before the blood was studied.

5. Goto, K.: Jour. Exper. Med., 1917, 25, 693.

Although most of these findings have been included in the preceding tables, particularly that of the marasmus cases, they are presented together in Table 9. In two children the blood was examined shortly

TABLE 9.

After Injection of Physiologic Salt Solution.

Name	Date	Age, Months	Injection	Water in Blood, Per Cent. by Weight	Total Solids, Per Cent. by Weight	Chlorid in 1 C.c. Plasma Computed as Gm. of NaCl
Salvatore L.....	Oct. 4	1	Oct. 3—90 c.c.	83.77	16.23	0.00523
John L.....	Oct. 4	9	Oct. 3—120 c.c.	82.73	17.27	0.00442
Thaddeus E.....	Oct. 4	7	Oct. 3—120 c.c.	83.63	16.37	0.00503
Grace D.....	Oct. 13	8	Oct. 12—240 c.c.	83.90	16.10	0.00515
John A.....	Oct. 6	6	Oct. 5—120 c.c.	81.40	18.60	0.00461
Seraphine C.....	Nov. 6	25	Nov. 5—240 c.c.	84.42	15.58	0.00618
Mary McG.....	Oct. 4	6	Oct. 2—240 c.c.	82.69	17.31	0.00626
Nathaniel D.....	Oct. 31	4	Oct. 30—120 c.c.	83.23	16.77	0.00644
Alfred D.....	Oct. 26	8	Before injection	(82.11)	(17.89)	(0.00576)
Alfred D.....	Oct. 26	8	Oct. 26—240 c.c.	0.00583
Albert H.....	Oct. 24	12	Before injection	(81.21)	(18.79)	(0.00483)
Albert H.....	Oct. 24	12	Oct. 24—240 c.c.	83.16	16.84	0.00539
Average.....	83.21	16.79	0.00545

before and again a few hours after the hypodermoclysis. All these children show a somewhat lowered average for blood solids, but it is not lower than the values found for several children in the normal group, and it is very near the average found in the pneumonia and the sclerema groups. The average for chlorid in the plasma after saline hypodermoclysis is approximately normal. Since all the children who received hypodermoclysis belonged to the abnormal groups, in which blood chlorid is invariably lower, it seems evident that the injection of the saline raises the chlorid content of the plasma. Of the two children whose blood chlorid values before the hypodermoclysis were obtained, the one who showed a normal value before, showed practically no change after the injection; while the one who was below normal before the injection was brought almost to normal by it.

It is difficult to draw any definite conclusions as to the effect of diarrhea on the blood solids and chlorid. Nearly all the children with

marasmus or intestinal disturbance had had more or less diarrhea. It might be expected that the blood water would be reduced, but this does not seem to be regularly the case. This group shows the lowest average found for blood chlorid. Except two cases in which soda bicarbonate had been injected, this group also includes all the very low values found.

An examination of Table 10 shows how great the tendency is for blood solids to remain constant. Only two groups show any signifi-

TABLE 10.

Summary of Tables.

	Water in Blood, Per Cent. by Weight	Total Solids, Per Cent. by Weight		Chlorid in 1 C.c. Plasma Computed as Gm. of NaCl	
		Range	Average	Range	Average
Gettler and Baker, adults. . . .	77.6	20.0-24.9	22.4	0.00500-0.00637	0.00592
Normal infants.	82.61	16.23-18.27	17.39	0.00536-0.00641	0.00587
Infants with pneumonia.	83.35	14.19-18.01	16.65	0.00432-0.00583	0.00505
Infants with tetany.	82.09	15.33-18.72	17.91	0.00458-0.00582	0.00512
Infants with marasmus, etc. . . .	82.94	16.10-18.63	17.06	0.00353-0.00576	0.00475
Infants with concentrated blood.	78.66	19.63-23.75	21.34	0.00318-0.00541	0.00452
Infants with nutritional edema	85.68	12.70-16.47	14.32	0.00437-0.00590	0.00499
Infants with sclerema.	83.48	16.27-16.77	16.52	0.00274-0.00644	0.00498
Child, 4 yrs. 8 mos., conva- lescent from infantile paraly- sis.	80.85	19.15	0.00594

cant variations from the normal. The children with nutritional edema seem to have a greatly diluted blood, while those with abnormally high hemoglobin have a greatly concentrated blood. All the other pathologic groups show blood solids not greatly different from the normal values for infants.

The most striking point brought out by this investigation is the fact that almost all children not in a normal condition have a remarkably low value for chlorid in the plasma. Only one of the normal children shows a value as low as most of the high values of the other groups. This child, H. R., though perfectly normal at the time of

examination, had suffered a year before from severe marasmus. Of the children in the pathologic groups that show values as high as normal, most had received a saline injection during the twenty-four hours preceding. A few other exceptions to the rule are found in some particularly well-nourished children in the pneumonia and tetany groups. It must be emphasized, however, that on the whole chlorid in the plasma is extremely low in children not in normal condition, especially if they are poorly nourished and suffering from digestive disturbances.

CONCLUSIONS.

1. The normal infants studied had about the same concentration of chlorid in the plasma as normal adults; the average for total solids in the blood was about 5 per cent. lower in infants than in adults.
2. In pneumonia there was irregularity in the values found, both for total solids and chlorid in the plasma, which is difficult to account for.
3. Children suffering from tetany showed a tendency to slightly higher total solids and higher chlorid in the plasma than was found in any of the other pathologic conditions studied.
4. Children suffering from malnutrition and digestive disorders showed the lowest chlorid in the plasma of all those investigated.
5. Children with nutritional edema all showed abnormally dilute blood, with chlorid in the plasma much below normal, though not lower than that of other sick infants.
6. Infants with sclerema had nearly normal total solids in the blood, with chlorid in the plasma like that of the other pathologic cases, excepting the marasmus group.
7. There was no constant relation between the variations in total solids of the blood and those in chlorid of the plasma.
8. Saline hypodermoclysis seemed to affect the percentage of total solids very little, but to raise the chlorid in the plasma nearly to normal.
9. In general, all infants not in normal condition had low blood chlorid, frequently very much lower than normal, but, except in edema, or under some unusual condition of blood concentration, the percentage of the total solids in the blood of infants tended to remain constant.

THE EFFECT OF COD LIVER OIL ON GROWTH IN A CASE OF "INTESTINAL INFANTILISM."*

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Greatly retarded growth, amounting to a condition of infantilism, is occasionally seen as a result of certain prolonged derangements of the functions of digestion, as well as a consequence of organic changes in these organs. The exact nature of the changes which bring about arrested growth in the first mentioned group of cases is as yet little understood, but it has generally been connected with disturbances in the metabolism of calcium, magnesium and phosphorus. Negative balances in these substances have repeatedly been found in children belonging to this class.

Because of the demonstrated effect of cod liver oil in increasing the retention of these inorganic salts in other conditions, particularly in rickets, it was determined to try its effect on the patient whose history is here reported, in whom the diagnosis of infantilism of intestinal origin was tentatively made.

Report of Case.

History.—Elizabeth R., aged 8½ years, came first under observation Feb. 9, 1916; her condition may be described in a word as "infantile," her weight being but 29 pounds net and height 39½ inches, or that of an average child of about 3 years. She was the eldest of three children, the other two being normal and well developed, a sister of 6 years being 4 inches taller and 12 pounds heavier than the patient. The parents were healthy and lived in a suburban town in excellent surroundings. The child had always had the best of home care.

Early progress was quite normal. She was born at term, weighing 8 pounds; was nursed for twelve months and did very well; muscular development was

* Read at the meeting of the American Pediatric Society, White Sulphur Springs, W. Va., May 28, 1917.

average. Weaning was easy and feeding for the second year not at all difficult. In fact, up to nearly 2 years of age, except that her stools were much of the time very pale, her progress was considered satisfactory. The food given at that time was chiefly milk, about 50 ounces being taken daily in five meals; she was also given beef juice, eggs and orange juice.

Ever since she was 2 years old the child had suffered from digestive symptoms, though she had never had acute attacks either of diarrhea or dysentery. Occasional vomiting was present during the first two years, but after that time vomiting attacks became more frequent, recurring every few days, and very often she would vomit during the night. The stools were always pale and were reported large in proportion to the food taken; at times she was constipated, but much of the time the stools were reported "frothy" in character, but no blood and rarely much mucus was present. There was always much gas in the bowels. Abdominal distention had been an almost constant symptom and most of the time it was extreme.

The diet had been much restricted. From the end of the second year milk had been excluded, also green vegetables, soups, cereals and fruits. Until a few months before her visit she had been fed chiefly on rare meat, eggs, toast and butter and a large amount of potato. For the last few months small quantities of milk had been added to the diet. The appetite was usually fair, but she constantly craved highly seasoned food and such articles as onions, strawberries, ice cream, lemons, green corn, etc.

The child had never had any acute illness except an attack of grip a few weeks before. Her progress mentally had been nearly that of the average child. On the whole, the mother considered her condition as rather better for the preceding six or eight months than previously, the abdominal distention had been much less and the stools of better character, but vomiting still occurred about twice every week.

No records of the weights had been kept; the following were given by the mother from memory:

At 12 months, 19 pounds; 3 years, 24½ pounds; 4 years, 30 pounds; 8 years, 30½ pounds (her best weight).

There were also no records of her height, but the mother thought that for the preceding two or three years there had been little or no growth.

Physical Examination.—On examination she appeared physically like an average child of 3 or 3½ years. The body was thin, but was in no sense wasted. The child was symmetrically small—trunk, face and extremities. The head was well formed and showed no irregularities or abnormalities; fontanel closed. Hands and feet, legs and arms were quite normal; facial expression, intelligent; speech and mental development were like that of an average child of 6 or 7 years. Physical examination of heart and lungs was negative. The circulation was rather poor; extremities much of the time cold. In the abdomen were found no abnormal masses; there was no distention. The liver and spleen were not palpable. Super-

ficial glands were slightly enlarged. The tongue was coated; the breath now, and usually, sweet; the temperature was normal. There was moderate beading of the ribs, and very slight epiphyseal enlargement, but no signs of active rickets were present. The teeth, twenty in number, were in poor condition; several were carious.

Measurements: Weight, 29 pounds net; height, 39 $\frac{1}{4}$ inches; head, circumference, 19 $\frac{1}{4}$ inches; chest, circumference, 20 inches.

Though she had suffered almost constantly from digestive symptoms since 2 years old, there did not seem to be in the diet a sufficient explanation for the arrested growth.

Metabolism Observations.—February 27 the child entered the Babies' Hospital for metabolism observations. She was given the same diet in variety and amount which she had been taking for several weeks previously. Though the quantity of food was small, it was all she would take at the time. The diet was as follows:

Breakfast: One egg; 2 slices toast; 2 gm. butter; 2 gm. sugar; one-half gm. salt.

Dinner: Scraped beef, 60 gm.; 15 c.c. beef juice; one-half small baked potato; 120 c.c. milk; 2 gm. sugar; 1 gm. salt.

Supper: Toast, one-half slice; 120 c.c. milk; 2 Huntley & Palmer breakfast biscuits; 2 gm. butter; 2 gm. sugar.

Was given 60 c.c. water at each meal and all she would take between meals.

Total calories in her food, 650.

Weight on beginning metabolism observations was 12.5 kilos (27 $\frac{1}{2}$ pounds); she was thus receiving at this time but 52 calories per kilo.

In Table 1 are given the average daily intake and excretion for the three days of metabolism observations.

On this restricted diet the child had relatively large, constipated, but well-digested, stools. The metabolism balances showed a fair nitrogen and fat reten-

TABLE 1.

Metabolism Observations, First Period—Feb. 23-26, 1916.

	Grams, Daily Average								
	Nitrogen	Fat	CaO	MgO	P ₂ O ₅	Cl	K ₂ O	Na ₂ O	Total ash
Intake.....	5.8398	24.176	0.5049	0.1802	1.3052	1.4279	1.4438	0.8593	5.4532
Excreted in feces.	0.7551	11.218	0.7043	0.1525	0.7219	0.0655	0.5075	0.0534	2.2425
Absorbed.....	5.0847	12.958	-0.1994	0.0277	0.5833	1.3624	0.9363	0.8059	3.2107
Excreted in urine.	4.2200	0.0181	0.0582	0.7149	1.2212	0.6523	1.0638	3.5802
Retained.....	+0.8647	12.958	-0.2175	-0.0305	-0.1316	+0.1412	+0.2840	-0.2579	-0.3695
Per cent. of intake									
retained.....	14.77	53.65	9.88	19.7
			-43.1	-16.9	-10.1			-30.0	-6.77

Dried weight of feces, average daily, 18.53 gm.

tion, but a very striking loss in mineral salts, only potassium and chlorin showing a positive balance. The abnormal loss took place chiefly through the feces.

For how long a period such a disturbance of salt metabolism had existed it was of course impossible to say, but the negative balances found coincided with the clinical history of the case. Something which would aid in the absorption of salts, particularly calcium and phosphorus, or lessen their excretion through the intestine was evidently what was needed. Even though there were no evidences of active rickets, it was decided to try the effect of cod liver oil as soon as the condition of the digestive organs warranted it, and at the same time to increase the salt intake by giving more food, particularly more milk.

At the end of a month the mother reported that the child had vomited but twice, that she was eating much better and was now able to take besides her other food, 360 c.c. of milk daily. The bowels were now quite regular. Cod liver oil with maltine (a preparation containing 30 per cent. oil) was begun about April 1, 1 teaspoonful twice daily, the dose being gradually increased until at the end of a few weeks she was taking 2 teaspoonfuls three times daily, this being equivalent to about 8 c.c. of the pure oil daily. This amount was continued up to the end of August, a period of about five months. It was then omitted for two months.

In June, July and August she suffered from a moderate attack of whooping cough. During this period the appetite was poor, but she "took her food as a duty," the mother reported. In spite of these adverse conditions the gain in weight was steady, so that by the end of August she was 5 pounds above her low weight early in March. With the subsidence of the whooping cough the appetite returned and her gain in weight was now rapid. The net weights during the summer were as follows:

June 9, 31½ pounds; August 25, 32½ pounds; September 28, 36½ pounds; November 1, 40 pounds.

The diet was practically the same as that mentioned above, except that the milk had been increased to 500 c.c. daily. Much difficulty was experienced in making her take vegetables; the only one she would take was carrots.

The next opportunity for an examination of the child was on November 10. Her measurements were as follows:

Weight, 41¼ pounds; height, 41½ inches; head, circumference, 19¾ inches; chest, circumference, 22½ inches.

This represented a gain in nine months of 2¾ inches in height and of 2½ inches in circumference of chest, and in weight of 13¾ pounds in eight and a half months. The improvement in the general condition was striking; though still very small, she was plump and well nourished; the tongue clean; the abdomen slightly distended; the circulation excellent. She was able to walk a mile a day.

For three weeks during the latter part of November with an attack of bronchitis, the appetite was lost and her digestion was considerably upset; she vomited occasionally; the bowels became loose and the same light colored "foamy" stools previously mentioned returned. In this attack she lost nearly 4 pounds in weight.

December 12 she entered the hospital a second time for metabolism study; observations were continued for a three-day period, beginning December 15. Her weight on admission was 37 pounds 6 ounces. The body was well nourished; color good; child good natured and happy; very much more active than during her previous admission. Her stools were formed, in fact, rather constipated, a daily enema being required; they were of a grayish-yellow color, but well digested.

She continued on the same diet which she had been taking at home, which was as follows:

Breakfast: One egg on 1 piece of toast, thinly buttered; 200 c.c. milk; 1½ slices graham bread and butter.

Dinner: Graham bread, 1½ slices, with butter; 2 tablespoonfuls scraped beef; 1 small baked potato with 1 ounce beef juice; 200 c.c. milk.

Supper: One slice white and 1 slice graham bread with butter; 200 c.c. milk.

Two teaspoonfuls of cod liver oil with maltine were, as before, given after each meal. Total calories given estimated at 1,030, making 61 per kilo or 28 per pound.

The intake excretion and balances for this metabolism period are given in Table 2.

TABLE 2.

Metabolism Observations, Second Period—Dec. 15–18, 1916.

	Grams, Daily Average								
	Nitro- gen	Fat	CaO	MgO	P ₂ O ₅	Cl	K ₂ O	Na ₂ O	Total ash
Intake.....	8.5701	43.382	1.3283	0.3821	2.6311	3.4650	2.3624	2.6159	11.9358
Excreted in feces....	1.4090	14.642	1.0994	0.3163	0.7450	0.1586	0.5841	0.2560	3.5217
Absorbed.....	7.1611	28.740	0.2289	0.0661	1.8861	3.3264	1.7783	2.3599	8.4141
Excreted in urine..	5.3390	0.0174	0.0513	1.3950	2.7840	1.0312	2.1192	6.7092
Retained.....	1.8221	28.740	0.2115	0.0148	0.4911	0.5424	0.7471	0.2407	1.7049
Per cent. of intake retained.....	21.3	66.3	15.9	3.87	18.6	15.65	31.6	9.2	14.3

Dried weight of feces, average daily, 31.5 gm.

This showed, as compared with Table 1, a somewhat increased retention of nitrogen and fat; but the most striking changes were in the salt balances. Instead of a negative balance in total ash of 6.77 per cent, there was a positive balance of 14.3 per cent. All the salts showed positive balances. The most marked changes were in the calcium where a negative balance of 43.1 per cent, was changed to a positive balance of 15.9 per cent.

Six weeks after leaving the hospital, in spite of intercurrent varicella and a mild otitis, the mother reported that she had never known her in better health; she had regained her weight lost during the bronchitis. A report late in March

stated that she was in excellent condition except slight stiffness of the legs (rheumatism?) which she had suffered from in previous cold seasons.

The effect of cod liver oil and phosphorus in increasing the calcium and phosphorus retention in rickets has been known for a number of years. The careful observations of E. Schloss,¹ Schabad,² Birk³ and many others⁴ have established this fact beyond question. It has been repeatedly noted in our own observations at the Babies' Hospital. In a recent case the figures for the per cent. of the intake of the different salts absorbed and retained were as follows:

	CaO		P ₂ O ₅		K ₂ O	
	Absorbed	Retained	Absorbed	Retained	Absorbed	Retained
Before cod liver oil and phosphorus.	16.6	14.0	51.5	18.0	74.2	12.9
After cod liver oil and phosphorus.	45.1	43.8	88.3	33.0	87.3	23.8

The value of the phosphorus in the combination is questioned by Schloss, though Schabad considers it important. Exactly how it is that the cod liver oil acts, has not yet been clearly shown. It certainly creates conditions which allow the calcium intake to become incorporated into the organism.

From Schloss' observations it would seem to be essential that there should be ample intake of calcium in order to obtain benefit from the cod liver oil. He found, for instance, that with a rachitic breast-fed infant the administration of cod liver oil and phosphorus did not improve the condition; but that when calcium acetate was added great improvement followed. It is undoubtedly true that the intake may be low in some breast-fed infants; but it is almost always adequate in those who are artificially fed.

In a later communication, Schloss states that calcium-phosphorus preparations alone with breast feeding are almost as effective in improving the calcium and phosphorus balances as when they are used

1. Schloss, Ernst: *Jahrb. f. Kinderh.*, 1913, 28, 694.

2. Schabad, J. A.: *Monatschr. f. Kinderh.*, 1911, 9, 659; 10, 12; 1912, 11, 4.

3. Birk, W.: *Monatschr. f. Kinderh.*, 1909, 7, 450.

4. Review of Rickets, *Berl. klin. Wchnschr.*, 1916, 50, 1340; 51, 1366.

with cod liver oil. The simple inorganic salts, he believes, added to breast milk as a food, work better than the natural salts of cow's milk, even when cod liver oil is added. His conclusion is that the nature of the food and the condition of absorption in the body are of even more importance than the form of salt addition.

In the patient under consideration there were but slight evidences of old rickets and none whatever of any active rickets. The cod liver oil was administered with the hope, but without very much expectation, that it might influence salt retention. At the same time, by increasing the diet, especially the amount of milk, an attempt was made to increase the salt intake. The results in this case far exceeded our expectations and they showed conclusively how essential to proper growth is the assimilation of the salts ingested.

There are certainly several factors that must be considered in explaining the improvement in this patient. An improved digestion from the omission of certain disturbing articles of food, an enlarged diet, particularly the addition of the milk, the cod liver oil, and possibly the preparation of malt with which it was administered—any one or possibly all of these were factors in the result. It is our own belief that the cod liver oil was by far the most important one, the next being the milk.

Whether children like the one whose history is here given are likely to reach a normal physical development, is a question of great interest. We know from the studies of Osborne and Mendel on rats that growth may be arrested by deficient feeding for very long periods, and then by proper feeding, growth and development may be resumed until the normal is reached. They found, however, that there was a period beyond which, if the deficient feeding was continued, growth was not resumed. There are, then, certain limitations to growth which cannot be exceeded. All children like this one need to be closely watched, and, if possible, metabolism observations made from time to time to determine the result of the feeding.

SOME OBSERVATIONS CONCERNING CHICKEN BONE MARROW IN LIVING CULTURES.

By RHODA ERDMANN.

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Tower and Herm¹ presented recently before the Society for Experimental Biology and Medicine some ideas concerning the origin of the mammalian (cat) and avian (chicken) blood cells. These authors were led by their observations on bone marrow in living cultures to the following conclusions:

1. The mammalian red blood corpuscle is a nuclear bud which escapes into the circulation as the true red cell.
2. The mammalian normoblast and the red corpuscle of the bird are the product of intranuclear activity and are phylogenetically identical.
3. Phagocytosis of red cells by the giant cells (megakaryocytes) in normal blood-forming tissues is by no means common. The true process is undoubtedly the manufacture of red cells and not the destruction of them.

My own observation of *chicken* bone marrow in living cultures led to some conclusions which are not in harmony with the quoted statement. I studied the bone marrow of *chicken*; therefore, my remarks are based only on observations on the bone marrow cells of this animal.

I could observe budding of red blood corpuscles after the first day of cultivation. Buds with and without nuclei appeared, also the rapid division of erythroblasts could be noted. The budding off of either small nucleated or non-nucleated cells cannot be a progressive process in the chicken because the avian red blood corpuscle is nucleated. Therefore the analogous observation of Tower and Herm with its conclusion that the mammalian red blood corpuscle is a nuclear

¹ Tower and Herm, 1916, PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 1917, Vol. XIV, pp. 51-52.

bud which escapes into the circulation as the true red cell, loses its convincing power; probably the budding is a reaction of the normoblast to the change of its media, as it is also observed in amebae as soon as they are under unfavorable conditions and can be produced experimentally. The mono- and polynuclear eosinophil leucocytes show in living cultures the same tendency to divide rapidly into nucleated *small* cells or non-nucleated components in living cultures. The bud-forming capacity and the tendency to divide rapidly seems to be a general behavior of blood cells in living cultures.

Another phenomenon to produce non-nucleated cells observed in living cultures is the following: the nucleated blood corpuscle loses its nucleus by *ejection* of chromatin, this process resembles the formation of Cabot's bodies in experimental anemia or the anemia of man (Juspa²).

These two processes seem to be *degenerative in the chicken*. If these two phenomena are observable in the chicken the question arises, can the mammalian normoblast be capable of losing either its nucleus by the budding off process or by ejection of the condensed original nucleus. The authors identify strongly in their second thesis the mammalian normoblast and the red corpuscle of the bird, it may be therefore possible that they have not observed the ejection of the mammalian normoblast nucleus.

I can agree with the authors that phagocytosis of true *megacaryocytes* (giant cells) is by no means common in normal bone marrow tissues. But still the most striking feature in *my cultures* was the phagocytosis of a kind of "Riesenzellen." But these "Riesenzellen" are not the usual multinucleated cells of the bone marrow; (megacaryocytes) this name includes cells which have first been observed in the bone marrow by Foot^{3, 4} and thought by him in his first publication to be of mesenchymal origin and in the second publication of lymphocytic origin. These "giant cells" can be either changed fat cells or elongated vacuolized connective tissue cells, or even enlarged myelocytes. They are able to phagotize, to store fat, to divide into

² Juspa, 1913-14, *Folia Haemat.*, Bd. XVII, II Teil, pp. 429-441.

³ Foot, *Beitr. z. path. Anat. u. z. allg. Path.*, 1912, Bd. 53, pp. 446-447.

⁴ Foot, *Jour. of Exp. Med.*, 1913, Vol. XVII, pp. 44-60.

smaller forms—the so-called cell culture type, small forms with nuclei, the chromatin of which is arranged on a fine network. The formation of “giant cells” characterizes the first period in the history of bone-marrow in living cultures. After five or eight days’ cultivation, the “giant cells” have cleaned up the debris of the dying cells (blood corpuscles, fat cells, and large mononuclear lymphocytes). In the second period the remaining cells adjust themselves to the continued life in tissue cultures. Cell types of the small lymphocyte type with vesicular nuclei appear, which later are transformed into different types of connective tissue cells—not exactly resembling the connective tissue cells in the outgrown animal—but closely resembling the mesenchymal cells of the embryo.

The production of the cells of the second period can be accelerated by washing the original bone marrow particle in plasma. After the plasma has been renewed three or four times, blood corpuscles and the lymphocytes which had been from the beginning in the meshes of the bone marrow, have been left in the media and only those cells close to the bone marrow network are transferred to the new culture medium. In cultures prepared in this way, we can observe small cells of lymphocytic character which can store fat, phagotize and adopt all shapes of connective tissue cells—but no formation of *blood* corpuscles or large mononuclear lymphocytes can be observed. This proves clearly that after the already preformed “ripe cells” are disposed of, no new formation of blood corpuscles takes place in the living cultures. It may be that the lack of oxygen prevents the appearance of red blood corpuscles. The conditions in tissue cultures do not seem to allow the stem cell to show its dualistic character. It does not form blood corpuscles, but forms only the different elements of *connective* tissue.

The above mentioned authors do not state in their preliminary paper how old their cultures were when they made their observations and if they have distinguished between cultures of fat containing bone marrow, nearly fatless bone marrow and bone marrow with large amounts of red blood corpuscles. All these facts may alter the conclusions because if the bone marrow contains a large amount of fat then many “Riesenzellen” are present and phagotization can be observed in a very considerable degree. If fatless bone marrow is

used, phagotization appears to go on in a remarkable degree only in the second outlined period of culture life, because only then the cells near the bone marrow network begin to migrate in the plasma clot, to phagotize and to assume different types of connective tissue cells.

CYTOLOGICAL OBSERVATIONS ON THE BEHAVIOR OF CHICKEN BONE MARROW IN PLASMA MEDIUM.

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The writer, employing the bone marrow of the chicken for attenuating the virus of cyanolophia (Erdmann '16¹), by culture of the marrow and the virus in a medium of chicken plasma, has observed some interesting facts concerning the cytological changes in the bone marrow cells.

The morphology and development of chicken bone marrow and its relation to blood formation have been described by few authors. Dantschakoff ('09, pp. 859-65) gives an extensive review of the literature on these questions and establishes our knowledge of the origin of the different elements of chicken bone marrow.

In studying the cells of bone marrow in plasma culture medium, we must take into consideration the fact, that we add to the plasma in which the tissue culture is cultivated a heterogeneous mixture of highly differentiated cells. Chicken bone marrow has a loose framework of slender connective tissue cells, in the meshes of which blood and fat cells are scattered. The blood cells—eosinophils, erythrocytes, and myelocytes—form, according to Foot ('13, p. 45) strands and circles between and around the fat cells. The blood islands represent collections of cells of microlymphocytic and macrolymphocytic types, of more or less ripe erythrocytes and of young connective tissue cells. It must be clearly kept in mind that all these different elements behave differently in the tissue cultures and may, after

¹ Erdmann, Rh. 1916. Attenuation of the living agents of cyanolophia, *Proceedings of the Society for Experimental Biology and Medicine*, vol. 8, pp. 189-193.

having undergone important changes in the plasma, offer some difficulties in interpretation.

The only observations of normal chicken bone marrow in plasma are those made by Foot '12 and '13. In the first series of experiments he studied especially the behavior of the fatty elements of chicken bone marrow, recording the following results. Six hours after implantation numerous cells leave the tissue center. They form rays of cells liquefying the plasma. These rays are formed by polymorphous leucocytes with eosinophile granules and by "eine Art von mononukleären basophilen Zellen" (p. 450). Foot gives the latter the name of X cells; they are the most important and they contain only fat according to his observations of 1912. They form, he says in 1912, the bulk of all cells migrating into the surrounding plasma. These X cells, the origin of which Foot tries to elucidate, are true phagocytes. They include small fatty droplets and other particles which are dispersed in the cytoplasm. On the fourth day, these cells, after having been enlarged by the amount of fat which they have taken up during the first three days in the culture, form either syncytial masses or a widely spread network of anastomosing cells. The former may divide, after having lost most of their fatty granules, and form the final 'ruhende X Zelle' (Foot '12, fig. 8, pl. 22): or the latter, after having been highly-vacuolized, as stated by Foot '12, may form fibrils (fig. 18, pl. 22). If these X cells do not form resting X cells or cells which produce fibrils, they take the shape of 'Riesenzellen.' These 'Riesenzellen' are not identical, in Foot's opinion, with the 'giant' cells of the normal bone marrow. They are represented in his figures 11, 16, 17, 19. They are only X cells which have fused together, form no fibrils, and may later break up in small cells (figs. 12 to 14), which have generally one nucleus. "Das Ergebnis der Aufteilung der Riesenzellen ist sozusagen eine neue Zellrasse" (p. 460)—cells adapted to the condition of the medium.

Foot believes that the X cells are transformed cells of the 'mesenchyme' and "Zwar indifferent gewordene Mesenchymzellen" (p. 466). He reasons as follows: Because these cells have the potentiality of forming fibrils they must belong to those cells which can form connective tissue, and therefore these X cells without any intermediate stages take their origin from mesenchymal or endothelial cells. In a

postscript to this paper he changes his opinion entirely and says (p. 475): "Was die Herkunft der X Zellen betrifft, so scheint es als ob die Hauptmasse derselben entweder direkt oder indirekt von den lymphocytären oder myeloblastischen Elementen des Knochenmarkes abstammte," promising to give the reasons for this change of opinion in his second communication.

After a careful study of Foot's second publication ('13), which is rather difficult to understand because he does not very often connect his first publication with the second, I restate in his own words his revised opinion of the origin of those cells which form X cells ('13, pp. 46-47). "The deductions as to the transformation of the lymphocytes from one form to another, which form the basis of the following descriptions, were made from the observation of transition forms. The later transformations of these cells into forms resembling fat and giant cells or cells of the connective tissue have been considered in my previous article." So it appears that the so-called X cells of this author ('12)—the name does not often appear in the paper of 1913—are not directly transformed cells of the mesenchymal type but are said to be of lymphocytic origin. He observes that as early as three hours after implantation of the bone marrow a considerable number of microlymphocytes emigrate from the tissue particle. Their transformation occurs in the following way:

The small microlymphocytes are first transformed into macrolymphocytes, later into large mononuclear forms, then into myelocytes. At last the polymorphonuclear leucocytes appear, after having undergone different changes in the form and structure of the nucleus. The nucleus is at first horseshoe-shaped, later polymorphonuclear and even polynuclear. Finally the cells, by rounding off and dechromatization of the nucleus coincident with the rarification and a change in the staining properties of the plasma, are transformed into the cell culture type (p. 56). This cell culture type (see his fig. 2, pl. 3, and his fig. 3, pl. 4) represents small polymorphonuclear leucocytes (p. 49) which have undergone the transformation, but not only does the cell culture type originate from lymphocyte forms, but this 'stem cell' can also be transformed through the transition stage of amoeboid forms into 'giant cells,' syncytia, and, as said before, into the cell culture type (table 1, p. 56).

Thus it is clear that, according to this author's view, all the different forms described by Foot in 1912 and 1913 originate from the microlymphocytes. Until the present time ('16) this important fact lacked verification, but by the cultivation of the virus of cyanolophia in chicken bone marrow an opportunity was afforded of observing the changes which Foot describes. A careful study of the morphological and cytological characters of the cells figured in the above mentioned papers, soon showed a lack of transition stages, which are needed as proof of Foot's final theory. Further, the nuclei of cell forms which are said to be transformed into each other do not show close resemblances, e.g., the cells in figures 1 and 3, 1913, which are said to be eosinophil leucocytes at different stages of incubation, have different nuclear structure as well from each other and from the cell of the cell culture type (fig. 2, left side, 1913). The nuclear structure of this particular cell (fig. 2, left side, 1913), however, has a certain resemblance to the nuclei shown in 1912, figures 5 and 6. These cells are considered by Foot as stages connecting the 'Riesenzellen' with "eine Art von monnukleären basophilen Zellen" (1912, p. 450). But here, as far as could be judged from the drawings, the cytoplasm of the cells in figures 5 and 6 is very different. Figure 5 has granules, figure 6 does not show them; only traces of digested nuclei of other cells are visible. These contradictory facts present *a priori* difficulties in accepting the views of Foot. But they appeared far more disconcerting on examining the cells themselves.

Technique of Cultivating, Preserving, and Staining Bone Marrow.

It is not necessary to describe in detail the technique of these cultures, since the writer followed the same methods as those used by Harrison ('10), Burrows ('11), and particularly Foot ('12 and '13). For storing the plasma it was deemed important to use the methods described by Walton ('12) for keeping mammalian plasma in good condition for long periods of time. Great stress was laid on the study of the living cells, and a warm stage was used to follow out the transitions of one cell form into another. The bone marrow of very young chickens, those of medium age, and of old individuals was studied; observations were also made on bone marrow which contained a very small amount of fat, as well as that which had a large amount of fat.

The method described below gave the best results in identifying and showing the stages of the individual cell types in stained preparations. A small particle of bone marrow was put into the plasma medium. The cells in the tissue were then allowed to migrate out of it. At periods of either 2, 4, 6, 12, or 24 hours, the original particle of bone marrow was extracted, and the fate of those cells which had emigrated was studied. The writer found that from the original particle of tissue numerous cell-forms had been sent into the surrounding plasma clot. Having thus extracted the bone marrow, it could be determined with absolute exactitude which cell-forms emigrated first, and the history of those cell types which had emigrated after 2, 4, 6, 12, or 24 hours, or at any given period, could be recorded. The extracted particle of bone marrow was now transferred to a new plasma medium and the cell forms which emigrated after the transfer were also observed. This was repeated several times, until practically all emigration of cells into the surrounding plasma had ceased. The structure of the remaining particle of bone marrow was of course studied. Smears and sections were made at every stage of the emigration process and a more complete history of this complicated process was thus obtained.

In staining the pieces of bone marrow, the methods used by Foot in 1912 and 1913 were followed and other methods for the discovery of fat were added (see descriptions of plates, page 118). Besides these, the Giemsa stain after moist fixation according to the prescription of Giemsa proved to be very satisfactory. No dry smears of bone marrow were used.

The Fate of Living Bone Marrow Cells Implanted in Plasma at 38°C.

The experiments from which the drawings on plates 1 and 2 were made were started on December 25, 1915, and on January 3, 1916. The bone marrow was taken from a full-grown chicken which had a large amount of fat, so that the pieces of marrow have a yellowish-white appearance. The first cells to leave the tissue after 40, 60, and 90 minutes incubation are, as Foot rightly remarks in his publication of 1913 (p. 49), small mononuclear or larger polymorphonuclear leucocytes (fig. 1). The forms have a very dark, granulated cytoplasm and are actively amoeboid (fig. 1). Pale mononuclear forms

without granulations but with their characteristic vesicular nucleus, follow closely the emigrating polymorphonuclear leucocytes. The fourth cell from the left (fig. 1) represents an erythroblast. The structure of the nucleus makes this evident. Besides these forms figured in figure 1, red blood corpuscles and a few fat cells were present in those parts of the plasma clot which surround the implanted bone marrow particle. The network of the bone marrow was injured by the process of cutting and tearing the particle into small pieces, and it is therefore not surprising that a large number of red blood corpuscles and some fat cells were scattered into the surrounding plasma clot. They are not figured in figure 1.

After 24 hours various other cell types have migrated into the surrounding plasma.

Figure 2 shows bone marrow which has been in the plasma for 24 hours, from January 3 to January 4, 1916. We can easily distinguish two different kinds of granulocytes: big cells which have round, shining granules, the nucleus nearly half as big as the cell and half-moon-shaped; and smaller forms, with very dark granules, the latter not rounded but more rod-shaped, the nuclei spherical and very often dividing. It is impossible to define without doubt the exact type of these granulocytes before the relation of their granules to basic or acid stains develops the true character of these cells. Therefore we do not venture any interpretation of the bigger type of these granulocytes but point out only that the smaller forms must be eosinophil leucocytes after their morphological structure, though their granules appear rather darker than those in non-incubated leucocytes of chicken-bone marrow. Also they have less distinctly round or less rod-shaped granules. These two observations are important. The big cell in the center of the figure 2 does not contain any granules but is from the large nongranular mononuclear lymphocyte type. Very often these cells break into pieces during observation.

Two other cells, one on the right, the other on the left side of figure 2 are of a different type. They contain large shining droplets, the fatty nature of which seems doubtless. Their nuclei have a vesicular structure and appear at this stage of the culture as often dividing. They are less numerous than the eosinophil leucocytes which form, in the first 24 hours, the bulk of all cells migrating into the surrounding plasma medium.

Figure 3 represents bone marrow which has been incubated for 48 hours (January 3 to January 5, 1916). Here a 'Riesenzelle' is rapidly moving; its cytoplasm is spread over a great area on the cover-glass and contains fat droplets and glistening granules. This 'Riesenzelle' shows in its cytoplasmic structure a close resemblance to the fat droplet containing cells on figure 2. To account for the larger size, we can either suppose that several of these cells have fused together or the cytoplasm of a single cell is thinned out by the method of cultivation.

The structure of the granulocytes is not very much changed. The larger forms with glistening granules and half-moon-shaped nucleus have diminished in number but smaller cells of the same type can be discovered now and then. In these forms sometimes fat droplets are visible. The eosinophil leucocytes are still abundant, but are surpassed in number by small ungranulated cells. These form now the bulk of the cells migrating into the surrounding plasma clot from the implanted tissue particle. They have either vesicular, less refractive or very shining and highly refractive nuclei.

In plate 2 we can follow in detail the further changes of the 'Riesenzellen.' The bone marrow (fig. 4) has been implanted 72 hours, from January 3 to January 6, 1916. Three round cells with big fat droplets can be seen, which seem to protrude out of the cell or cover its surface. The nuclei are therefore very seldom visible. When visible, they appear dark. A few granules are contained in the cytoplasm besides round or irregularly shaped masses, which seem to be remnants of other cells. On the third day after implantation these cells immediately attract the attention of the observer. They seem to have taken the place of the 'Riesenzellen;' this could be demonstrated by observation of the living cells. Some 'Riesenzellen' break apart, take on a round shape and completely extrude the fat droplets. These may be small or larger (fig. 5, second cell, left side) and show very fine pseudopodia. They are round cells which can survive an indefinite time in the plasma medium, the so-called 'cell culture type.'

Many 'Riesenzellen' however (fig. 5), the similarity of which to the round cells seen in figure 4 can be easily discovered, show all signs of degeneration. The cytoplasm has a 'curdled' appearance and is torn. The fat droplets have been thrown out into the plasma clot, and the

granules have acquired a dark appearance. This regressive process takes place on the fourth or fifth day after implantation. These decaying cell masses are surrounded by small granulated and ungranulated cells and seem to be able to phagotise, because their cytoplasm shows in some places 'curdled granules.'

During the next days of incubation, no striking changes take place. The number of living cells diminishes and few types of cells are in healthy condition.

Fig. 6 shows cells which have been incubated in the same plasma medium 216 hours (from December 25 to January 3). They have small distended nuclei which do not seem to contain much chromatin, and the cytoplasm is filled with shining droplets. They belong to the so-called 'cell culture' type. Besides these cells we find others with oblong nuclei and elongated cytoplasmic bodies full of glistening fine granules. These move slowly and show fine pseudopodia formed by their delicately granulated cytoplasm.

To summarize: Fat containing bone-marrow of chicken when incubated for 9 days in a plasma medium, undergoes the following changes which can be observed in the living preparation: The signet-like fat cell disappears, it is transformed to 'Riesenzellen' and finally to the 'cell culture' type. This type includes round cells with coarsely granulated cytoplasm, big shining droplets and oblong, less refractive nuclei. The other prevailing cell-form is distinguished by its finely granulated cytoplasm, elongated or round cell body, and oblong nucleus.

These two cell types (not widely different in their morphological bearing) are always to be found among the cells which have migrated from the implanted bone-marrow particle into the plasma clot. Besides these cell forms,—capable as it seems of metabolism for long periods,—we see all forms of disintegrated cells. The cytoplasm and nucleus separate and the preparation is filled with débris. Fat droplets of different sizes which are freed from the cell fill the preparation. Nuclei of small granulocytes and lymphocytes without cytoplasm are often seen. Also shadows of blood corpuscles and granulocytes of all sizes are present.

It is certain that in non-renewed tissue culture retrogressive and progressive processes take place. It will be necessary to investigate

the more intimate phenomena of these changes in stained preparations specially adapted to the study of each different cell type by different methods of cultivating and staining.

The Fate of the Mononuclear and Polymorphonuclear Eosinophil Leucocytes of the Bone Marrow in Tissue Culture.

While describing the changes of the *living* bone-marrow cells after they had been 1, 24, 42, 72, 96, and 216 hours in the plasma medium, —the present author could give little or no definite interpretation of the changes observed in the different types. Some exact knowledge could be acquired only by comparing and combining the phenomena observed in bone-marrow cells in preserved and stained preparations after they had been in the plasma medium for well defined periods.

In figure 7, an exact microscopic field of a bone marrow preparation, after 36 hours incubation, is shown. The implanted tissue particle would be (if shown on the drawing) on the left side of the preparation. The cells shown have migrated to the zone next to the implanted bone-marrow tissue particle which was taken from a full-grown chicken and contained fat.

Eosinophil leucocytes in various developmental stages are numerous. They are in rapid amoeboid movement, and by continued fragmentation diminish in size and multiply in number. Their plasma is slightly basophil. The nuclei are strongly chromophil and the nuclear leucocytic structure in most forms is indistinctly developed. By comparing the nuclear structure with that of eosinophil leucocytes which have been 24 hours in cultivation (fig. 9) we can better distinguish the typical leucocytic network of chromatin particles and threads. The plasma of these leucocytes and of those figured in figure 8, which have been only one hour in the plasma medium, is acidophil and the round granulations are very distinctly recognizable.

Besides the changes in the cytoplasm of the leucocytes from acidophily to basiphily, other phenomena are noticeable. After one hour and still more after 36 hours incubation, the leucocytes of all sizes are losing and expelling the granulations. The nuclei of these forms have either become pale and indistinct (fig. 7, right side, below) or condensed and strongly chromatic (figs. 12 to 14). They may fade out to mere shadows and disappear.

The farther the polymorphonuclear eosinophil leucocyte advances into the plasma clot, the more its cytoplasm spreads out in the tissue culture. The granulations in consequence no longer appear lying closely together, but seem widely scattered in the cytoplasm. The leucocytes finally lose their power of cytoplasmic division. This happens generally on the margin of the plasma clot where the culture medium is thinly spread. The horseshoe—or kidney-shaped nuclei separate, become pyknotic and form round, chromatic bodies (figs. 11 to 19). The acidophil granules become more and more indistinct, the cytoplasm is again acidophil, and partly vacuolized. In this stage, long chains of these forms closely lying together cover the outer zones of the preparation, giving it a reddish halo. Later these cells without granules flatten out entirely, lose their nuclei or their chromatic particles, and undergo total destruction.

To summarize: most mononuclear and polymorphonuclear eosinophil leucocytes with either round, kidney-shaped, or lobulated nuclei, during the first hour of their emigration (fig. 8, and fig. 43) into the surrounding plasma, divide rapidly. They form smaller cells with fewer granules and a more basophil cytoplasm. Later by dividing and moving to the outskirts of the plasma clot, they finally form rays and layers of partly acidophil, vacuolized 'cells' without nuclei and granules. Another group of these eosinophil leucocytes, before diminishing in size in the zone near the implanted bone-marrow particle, had extruded its granules at a very early period. They fade out and leave their more basophil cell bodies in the plasma clot. The mononuclear or polymorphonuclear eosinophil leucocytes undergo a regressive development in tissue cultures.

These conclusions agree with the writer's own observations of the cells in living preparations. On the first and second day of incubation the eosinophil leucocytes are numerous and of normal size (fig. 2, left side, above). On the fourth and the fifth day the few forms, which have not undergone the flattening-out process and which have not changed their character, are small, with fine granules and an ellipsoid nucleus (fig. 5, left side, below). Foot ('13, pp. 49-51), in his account of the changes of the eosinophil leucocyte in the culture medium, reports that these cells finally take on the same form as

that assumed later by the large mononuclear lymphocytes, and cannot be distinguished from them. With this conclusion the present writer cannot agree. In figure 8, the emigration of small leucocytes is shown. The lean, almost fat-less bone-marrow originated from a young, not full-grown chicken. After an hour in an identical preparation the tissue was extracted and only the emigrated cells were allowed to develop. All cell types which are pictured in figures 11 to 26 are cells which have emigrated early from the bone-marrow particle, advanced to the border of the plasma medium, and changed in different ways.

Figures 11 to 19 show the regressive development of the polymorphonuclear leucocyte which is inserted in the plasma, either as a younger form, with spherical nucleus, or as an older form with kidney—or horseshoe-shaped, or lobulated nucleus always recognizable because of its acidophil granules. The long chains of these deformed cells in all transitions are easy to identify in preparations, where only a few cell types have been allowed to emigrate into the plasma. Here they never take on the character of the 'cell culture type' (Foot).

When bone marrow is taken from a young, poorly fed chicken and treated as above described, few 'mononucleäre basophile Zellen' emigrate in the first half hour, and the bulk are only eosinophil leucocytes (fig. 43). If these preparations are allowed to develop two or three days the rays of cells consist for the most part of these eosinophil leucocytes and few X cells or forms of the cell culture type are visible. If the process of extracting and again implanting the bone-marrow particle is repeated and the cells of the succeeding emigrations are controlled, few eosinophil leucocytes are observed in the second and third stage and after the third implantation approximately no eosinophil leucocytes are to be seen.

Therefore, no new formation of this cell type from a stem cell could be observed in the plasma clot, but only a process of emigration, multiplication, transformation and degeneration of those forms which were implanted with the bone marrow in the plasma clot.

*The Fate of the Erythrocytes and the Erythroblasts in the Bone Marrow in Tissue Culture.*²

The general rule for the behavior of cells in tissue culture: the more they are differentiated, or adapted to certain functions, the quicker they undergo destruction holds true in the case of erythrocytes. The red blood corpuscles appear often without a nucleus or without a shadow of a nucleus. The plasma seems perforated. This indicates that the haemoglobin has disappeared. Those cells in which we can trace only the shadow or a faint remainder of the nucleus are apt to deceive the observer. The remainder of the nucleus appears like a small parasite but is nothing more than the nucleus of the cell, as can be proved by numerous intermediate forms. These bodies resemble the Cabot's bodies which are described by Juspa ('14, p. 429) in certain diseases of men. Also the nuclei may become pyknotic in other forms and the plasma may disappear. Foot ('12, p. 461, and 1913, p. 46) notes these same two different ways of degeneration in erythrocytes. Their dead nuclei or their plasma is often incorporated into phagocytic cells (figs. 34 and 35) the origin and types of which will be discussed later.

The non-elongated round or irregularly shaped erythroblasts have a pale yellowish or colorless plasma (figs. 1 to 3). Well developed erythroblasts are distinguished when stained by their wheel-like, highly chromatic nucleus. Unstained cells show a whitish appearance of the nuclear membrane which seems crowded with the content of the nucleus and ready to break. Figures 3 and 7 represent erythrocytes and erythroblasts in various stages of their retrograde development. Their plasma-less nuclei cover the microscopic field and are often seen incorporated into cells of phagocytic character. Unripe, young erythroblasts are figured in figure 8. They have larger nuclei in proportion to their basophil plasma than the erythrocytes and are scattered, through the tearing apart of the bone-marrow network, in large quantities into the surrounding plasma. They are recognizable in stained preparations by the smooth surface of their plasma

² Erdmann, Rh. 1917. Some observations concerning chicken bone marrow in living cultures, Proceedings of the Society for Experimental Biology and Medicine, vol. 14, pp. 109-112.

and their chromatic nuclei and cannot be confused with "eine Art von basophilen mononucleären Zellen" which, according to Foot '12, form the X cells and the cell culture type.

But the difficulty begins when very young, i.e., small cells characterized in the first day of incubation by their situation near the bone-marrow network, are to be isolated and cultures from young living erythroblasts and from young basophil cells with vesicular nuclei are necessary, for deciding different questions. My experiments only proved, after isolating young cells near to the bone-marrow network that they underwent no transformation into erythroblasts but showed the phenomena fully described later on page 621-626 the transformation into cells of connective tissue cell type. It is naturally not excluded that erythroblasts—when they are already erythroblasts in a strict sense—divide in the tissue cultures, but I never could isolate this cell type with any certainty just at the point in being transformed from its 'stem cell' into erythroblasts. This phenomenon seems not to take place in tissue cultures.

The Fate of the Implanted Microlymphocytes in Tissue Cultures of Bone Marrow.

The microlymphocytes in chicken bone marrow are found in great quantities. Their small protoplasmic brim and condensed, highly chromatic nuclei allow us to distinguish them easily from the small basophile round cells with vesicular and achromatic nuclei, closely situated to the network of the bone marrow. The microlymphocytes seem to be present in the tissue cultures from the first day of the incubation of the bone marrow, without apparent changes, until the last day of cell life in the culture. But are those the same forms which were incubated or newly originated forms? The microlymphocytes implanted with the bone marrow particle must be capable of active movements, because they are no longer visible in the meshes of the bone marrow network after several days' incubation, but are always present in the plasma clot. In the preparations where only a few cells are allowed to emigrate and to stay several days in the plasma medium, the microlymphocytes are widely scattered. Their own cytoplasm expands in a star-like manner, often forming long cytoplasmatic rays. After a fortnight in the culture medium, they have the

appearance of forms such as the cells pictured in figure 25. One cell appears normal; the other has a torn cytoplasmic body. Figure 27 shows the remaining nuclei which will soon undergo complete destruction. Foot '13, page 43, believes that besides numerous microlymphocytes, which die, a large number 'steadily increase in size' and either form cells of the macrolymphocytic type or of the large mononuclear lymphocytic type, "after the latter has undergone nuclear enlargement and dechromatization." Foot presents no drawings of these highly important forms, but considers it sufficient to record the measurements of microlymphocytes of different sizes, measuring from 3.5 to 9.6 in diameter. The nuclear structure of these transition forms is not described by him. The present author has never seen cells with typical microlymphocytic condensed nuclei in all sizes, only cells with vesicular achromatic nuclei in every possible size. In the later discussion these contradictory reports of Foot and of the present author must be borne in mind.

Some authors hold the theory that microlymphocytes originated from the large mononuclear lymphocytes by multiple simultaneous divisions. Only in very recently incubated tissue cultures, as recorded on page 607 a breaking of large lymphocytic forms into pieces was observed. But the isolated cultivating of these small cells afforded no definite results. Multinucleated forms with ragged or torn cytoplasmic structure and nuclei with highly condensed chromatin may be observed in the case illustrated, of which three have a condensed chromatic structure (fig. 8). The younger the implanted bone marrow is, the more numerous these forms appear to be. They have a slight resemblance in their plasma to very young connective tissue cells, as, e.g., Maximow ('10) pictures them in figure 43, from a guinea pig, but they seem to have no connection with the formation of bone marrow lymphocytes.

To summarize: The microlymphocyte belongs to those cell types which undergo no progressive development in the tissue culture.

The Fate of the Implanted Myelocytes in Tissue Cultures of Chicken Bone Marrow.

From the first to the sixth day after incubation large cell types can be observed in the tissue culture of bone marrow when the experiment is conducted with a full-grown, over a half year old chicken. These cell types have, as described on page 79, before staining and preserving, a half-moon shaped, or elongated nucleus, and their plasma is either granulated, or the granules are invisible during cell life. The cells shown in figure 2, two granulocytes and one ungranulated large cell, have only been one day in the culture. The first type appears to divide; we can observe smaller forms on the following days, with larger granules than the eosinophil leucocytes possess. The other represented cell type is a large lymphocyte. These forms may break in pieces during observation. After six days incubation we discover in stained preparations the changed form of the myelocytes (figs. 39 to 42). The reddish ripened nucleus of these forms has all the characteristics of a myelocytic nucleus. But in eosinazur stains such nuclei are generally supposed to have a more bluish color. This must be explained by the rising acidity of the culture medium in growing tissue cultures (Rous, '13, pp. 183-86). The cells in figures 39 and 41 must be considered eosinophil myelocytes, those in figures 40 and 42 mononuclear lymphocytes. In earlier stages of their degeneration process these large forms often have very fine acidophil granules in their cytoplasm when observed on the second or third day of incubation; but they are never seen to divide. Their plasma loses its granulations, flattens out, and vacuolizes. The eosinophil myelocytes and lymphocytes have only a regressive development in the tissue culture medium.

The Fate of the Fat Cells of the Bone Marrow in Tissue Culture.

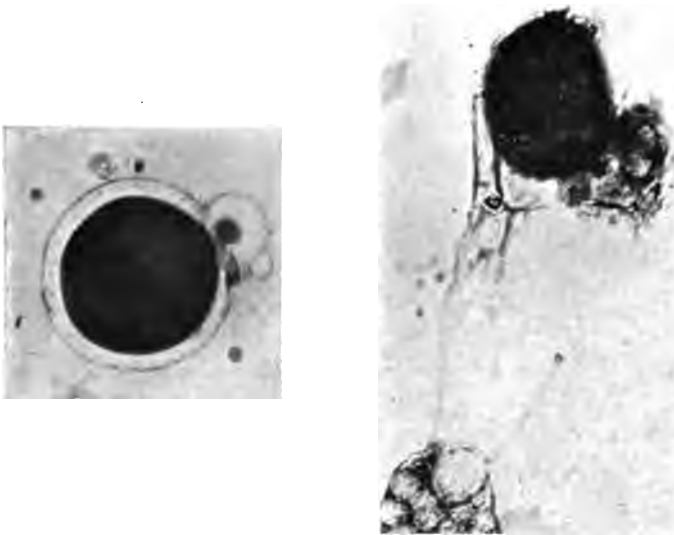
But one observation of the behavior of fat cells in tissue culture is given by Foot, who writes ('12, p. 447,) that the cultivation of subcutaneous or subepicardial adipose tissue was without success, growth of considerable amount could not be observed. The present writer repeated Foot's experiments. Adipose tissue of the omentum of the chicken showed, after three days incubation, almost a complete dis-

integration; further, the formation of few cells of the 'cell culture type' and the survival of connective tissue cells could be observed. It may be conceived that some connective tissue cells may have originated from fat cells losing their fatty contents and assuming the character of the known type of connective tissue cells. Or the connective tissue cells, implanted together with the adipose tissue may have developed and multiplied. This is a separate question which has not been sufficiently studied in true adipose tissue.

The changes of the fat cells of bone marrow in tissue culture, though not considered by all authors to be real fat cells, have a great resemblance to phenomena seen in rapidly growing embryonic adipose tissue, as Foot remarks (p. 48, '12). But he himself, neither in 1912 nor in his later publication of 1913, states the ultimate fate of the implanted, so-called fat cells, which, together with the other cells of the bone marrow, are in the culture medium and are numerous in the white bone marrow of the adult chicken. The typical signet-ring cell may apparently remain unchanged for 24 hours in the plasma medium, as it is shown on a photograph (fig. 46, right side, above). But the observed facts do not agree in most cases with this view. After three hours incubation all fat cells show still their accustomed shape. The big fat globule surrounded by a brim of cytoplasm flattens out and the large globule of fat separates into small droplets. Or the fat cell divides into two parts, and even a process of budding may be observed (figs. 29 and 30). If the cell has not divided up, the fat globule diminishes in size and does not fill the whole cell. With a specific fat stain it can be shown that the cytoplasm is filled with small fat droplets and strands (fig. 28). Later foam-like masses of cytoplasm, in the meshes of which the fat is easy to identify, protrude from the cell margin and separate themselves partially or totally from their 'mother cell.' Cells of this kind may offer the appearance of cells figured in figure 2, left side, in unstained preparations. In a tissue culture of 24 hours incubation, preserved with Orth's fluid and stained with Giemsa stain, they appear as cells with highly chromatic nuclei, and perforated cytoplasm (figure 7, right side and figures 33 and 34); also weblike masses, apparently without nuclei, are frequent (fig. 7) which are often surrounded by microlymphocytes and polymorphonuclear leucocytes. Text-figure A gives the most striking

phases of the activation of a fat cell. The original fat cell, the fat cell which has extended fine pointed processes, and the final stage that comprehends cells containing vacuoles which may still have traces of fat in them. (Compare cells on figure 2; figure 7, cell right side, above; and figures 45 and 46).

It must be kept in mind that these changes occur during the first 24 hours or 48 hours of incubation. Figures 45 and 46 show that in



TEXT FIG. A. Fat cells after 6 and 12 hours incubation.

a 30 hours culture the dissolving of the big fat globules and the dividing up of the fat cells has been in progress. The cells form chains, typical for the stage of the culture of 24 to 48 hours of fat containing bone marrow. These cell chains flatten out, fine processes are extruded which cover great areas and may fuse with other cells in web-like masses. Figures 45 and 46 give a good surview of this process and such a cell is also represented in figure 33. We note its enormous size, its big vacuoles, its slender processes, its phagocytic capacity and its small nucleus. In short, we see a so-called 'Riesenzelle' of Foot which is already present after 24 hours of incubation. Now Foot ('12, p. 459, fig. 5) gives the photograph of a preparation of

bone marrow after 5 days of incubation in a plasma medium. This is a discrepancy for which no explanation could be found.

It is of importance to state that all vacuoles do not contain fat in such a condition as to make it visible by the osmium process. The cell (fig. 32) shows still some fine traces of fat, but in many preparations which were treated with Scharlach or Sudan stain after adequate fixation, the vacuoles were devoid of fat. It is conceivable that fatty acids or other products of related character fill the vacuoles, but even after trying the most complicated stains (Ciaccio, Benda) to elucidate the nature of the contents in the vacuoles, no final decision could be reached.

From the third to the fifth day, the number of 'Riesenzellen' has diminished; we see smaller round or oblong cells with one or several vacuoles, with oblong faintly chromatic nuclei (fig. 34). They are the products of the breaking up of the 'Riesenzellen' and seem to be identical with Foot's cell culture type. They are capable of phagocytosis and move slowly toward the periphery of the plasma clot.

How can we interpret these extraordinary changes in the fat cells? The only similar observation was made by Maximow ('04, p. 108), describing the changes occurring in the cells of inflamed connective tissue of the rat. There he gives a good description of the involution of the fat cells. The process shows the same phenomena in the involution of the fat cells in the connective tissue of the living animal after inflammation as are to be seen in tissue culture. The flattening out of the cytoplasm, the dividing up of the big fat globule into small droplets inside the cell (Maximow, plate 3, fig. 9; Erdmann, text-fig. A) and the transformation of the plasma in a honeycombed mass (Maximow, plate 3, fig. 11; Erdmann, fig. 7, left side, above), are identical processes in both cases. Maximow believes ('04, p. 119) that some of these cells become fibroblasts. The present author ventures no opinion on the subject, though a striking similarity exists between the fibroblasts of Maximow (text-fig. B) and the cell in figure 7, right side above.

We find after the second day in our cultures: (1) cells of the fibroblast type; (2) cells of the 'Riesenzellen' type; (3) cells of the cell culture type, after Foot. All three types can originate from the implanted fat cell.

Besides these progressive changes we must state that many implanted fat cells undergo destruction. This is shown by the observation of the living cells as described on pages 605 to 609. Figure 4, shows such a disintegrating mass of fat cells from an unstained preparation, and figure 7, shows the mass in a stained preparation. Here two cells of the honeycombed type are recognizable (left side, above), one of which is intact, the other has expelled the contents of the plasma. Microlymphocytes are gathered around the disintegrating fat masses and the transformed fat cells. Maximow describes how his



TEXT FIG. B. Maximow, 1914, figure 8, plate 3. Involution of a fat cell in an area of inflammation into a fibroblast.

polyblasts, cells of the lymphocyte order, crowd around the fat cells and destroy them by phagocytosis (page 120). The same phenomenon occurs in the tissue culture; between the second and the fifth day the destruction and resorption of the dying fat cells is finished and the tissue culture gradually assumes a different aspect, as will be described later.

But together with these retrograde processes, easily observed in the living culture, small parts of the irregularly-shaped, large, disintegrating fat cells isolate themselves. They become spherical in shape and begin to wander away from their 'mother cells.' They can be recognized by their small nuclei, their coarse glistening plasma. They

are identical with small fat cells. This 'rejuvenation' of the fat cell was only observed when bone marrow tissue of younger well-fed animals was implanted. Bone marrow from very young chickens and tissue from old hens seldom rejuvenate the fat cells, when such are present. In tissue from older hens the disintegration of the fat cells often obscures the observation of the other cell types.

The Fate of the Mononuclear Basophil Cells of the Bone Marrow in Tissue Cultures.

When implanted in the plasma medium, the bone-marrow particle itself appears basophil after preservation with Orth's fluid and staining with Giemsa stain. For a long period, up to 14 days, it shows a strong basophilic character. We have shown how fat cells and their derivatives generally have a strongly basophil nucleus and often a basophil plasma. Erythrocytes, erythroblasts, and eosinophil leucocytes, which show a strong basophily of the nucleus, emigrate or are washed out of the tissue particle and either perish or undergo the changes described. The eosinophil leucocytes, diminishing the size of their nuclei and acquiring an acidophil cytoplasm, later form, together with the erythrocytes, the reddish halo around the implanted particle.

After the first emigration or washing out of the cell types mentioned, the tissue particle consists almost solely of basophil cells, which are very young, small, unripe erythroblasts, small lymphocytes, connective tissue cells of the bone marrow network, and basophil cells of all sizes and forms, the character of which is not at first recognizable. The thickness of the tissue particle prevents the closest examination, but these cells have always ungranulated plasma. In figure 8, a general survey of these basophil cells is given, as they appear after one hour's incubation in bone marrow of a young nearly fat-less chicken. Two types besides the erythroblasts with their more or less pinkish plasma and their wheel-like nuclei are distinguishable—cells with crude irregular cell plasma, as if it has been torn. They possess small, condensed, highly chromatic nuclei (fig. 8 left side, above), or their cytoplasm has well-rounded contours and a very big nearly chromatinless nucleus. This type and its changes will now be described.

In figures 11 to 27, different emigrated cell types of a similar bone-marrow particle are represented. The particle itself was twice extracted during an incubation period of 24 hours. The emigrated cells of each extraction stayed 12 days in the plasma until they were preserved and stained and later analyzed, so no new rear guard of eosinophil leucocytes and those mononuclear basophil cells, the fate of which Foot tried to elucidate, need be considered. According to this experiment, which was repeated several times, besides the eosinophil leucocytes the changes of which (figs. 11 to 19) have been fully treated on page 612, six different cell types are recognizable after the second extraction.

1. Cells which resemble fat cells (figs. 20 and 21).
2. Cells which, by their nuclear structure but not by their cell plasma, resemble true connective tissue cells (figs. 22 to 24).
3. Cells which are true connective tissue cells, from the type of endothelial cells (fig. 27).
4. Cells which are true connective tissue cells not shown in figures 20 to 27 but in figure 9, with star-like, fine protoplasmatic processes and elongated, often cone-like shapes, and a more mesenchyme-like character.
5. Cells which are microlymphocytes (fig. 25 and also fig. 27).
6. Cells which are lymphocytes (fig. 26).

Cell types 3, and 6 are not often found in preparations made according to the prescribed method. The lymphocyte with its fine red granules (fig. 26) shows all signs of degeneration. It appears highly probable that in the plasma clot the normal ripening out of the large mononuclear lymphocyte began but could not be fully accomplished owing to the conditions of the culture medium. The endothelial cell and the elongated connective tissue cells (figs. 27, 9, and 38) have not changed their characters. They already appear on the first day after incubation, because they could be observed in bone marrow culture of 24 hours incubation. The elongated connective tissue cell is highly amoeboid, and shows in its plasma, on the first days of incubation, fine and bigger fat droplets, which are coarser when stained with specific fat stains. Later their plasma looks as if pulverized with small fat droplets, still later they lose their fat and appear highly vacuolized. They repeat on a smaller scale the changes of embryonic

subcutaneous connective tissue that had been incubated 14 days in a plasma medium. Because these cells appear after the first day of incubation (the present author has observed them after but five hours' incubation) it appears highly improbable that they originated from the basophil spherical cells in question. They are cells of the bone marrow network or the vessels of the bone marrow, which have been torn apart by the cutting of the bone marrow. They can be also observed in tissue cultures of true adipose tissue and are distinguished by their rapid division rate.

In most cultures of connective tissue made by various authors these cells have been described. Lambert and Hanes ('11) mention the accumulation of fat and the vacuolization of the cytoplasm in cells of mesenchymal origin. They represent tumor cells in their publication of 1911, plate 66, figures 4 and 5, of this character. Lambert himself in 1912, on plate 72, figure 3 and plate 74 figures wandering cells from the chick spleen. Some of these forms are more related to the connective tissue cell type in question, some resemble more the cell type seen in bone marrow cultures when the fat cells have begun the disintegration. In 1914, plate 44, figure 6, he gives a good proof of this.

In figure 9, Carrel and Burrows, ('11), represent also fat storing cells of this type. They are said to be originated from an adult chicken spleen, while the first author must have seen the elongated vacuolized type ('13, plate 17, figure 16), in cultivated connective tissue. Lewis, R. M., and Lewis, H. W., '11, show on their figure 20, left side, in a chicken liver culture, highly vacuolized cells of the same type.

This comparison could be continued but the facts prove already that among connective tissue cells of the most varied parts of the chicken body these elongated, finely vacuolized, slender cells appear with a true connective tissue cell nucleus. They are all similar to the figures of Foot representing his X cells (cf. Foot '12, plate 22, figures 8, 16, 19). The connective tissue cell represented by the present writer in figure 9, is taken from a young chicken and is not of the same size as some of those cells which Foot shows. When cells, however, were taken from the bone marrow of a full-grown chicken, they were of the same dimensions as those given by Foot, '12, plate 22, figure 8.

Also, in the development of embryonic bone marrow tissue of the chicken, Dantschakoff, '09, depicts mesenchyme cells (plate 44, figures 5 and 6) which have a close resemblance to the above mentioned cell type (fig. 9). They are identical types, except that the latter may contain fat, the first are fatless. In this group must also be included the elongated forms of Foot's Riesenzellen which have pointed pseudopods.

To summarize: Though fat containing and often vacuolized the elongated cells with connective tissue like nuclear structure which appear in Foot's figures among his 'Riesenzellen' are true connective tissue cells. There can be no doubt that the granular lymphocytes, the elongated cells of connective tissue character, and the endothelial cells did not originate *de novo* in the tissue culture.

In studying the cells close to the connective tissue network of the bone marrow the present writer could only distinguish one well defined cell type (figs. 36 and 37). Small round cells with strongly basophil cytoplasm and large, faintly staining nucleus with two nucleoli are abundant. They are neither microlymphocytes nor mononuclear lymphocytes nor erythroblasts. They differ from the microlymphocytes by their vesicular nuclei, from the mononuclear lymphocytes by their size and their cytoplasm, from the erythroblasts by their nearly chromatinless nuclei and also by their size. In living cells the nuclei of erythroblasts appear whitish, the nuclei of these cells dark. If these cells, which migrate from the tissue particle after the leucocytes are washed out by continued changing of the plasma, on the second incubation are allowed to develop we find after a fortnight two different types: figures 20 and 21, and figures 22 to 24. The cell represented in figure 21 differs from the basophil cells which had been implanted into the tissue culture (fig. 8, and figs. 36 and 37) only by its size and by the more chromatic contents of its nucleus. These forms are numerous; they later contain fat or vacuolize, forming chains, the cells of which are always to be distinguished by their nuclear structure from the eosinophil leucocyte. The nucleus has a close resemblance to that in fat cells; it is vesicular with round, bulky, chromatic contents.

The next group (figs. 22 to 24) have a true connective tissue cell-like nuclear structure. The nuclei are elongated and fine threads of

chromatin form a true connective tissue nucleus network. The cytoplasm is basophil in most cases, but in certain parts of the culture and in very old cultures it becomes acidophil. The basophily or acidophily of cells is no constant character in tissue cultures. Rous ('13, page 183) points out the changes in acidity of growing cells. The cells themselves become acid in the culture medium, after having been basophil. Later they may regain their basophil character. The cells in question are true phagocytes (fig. 23). They contain fat, blood corpuscles, dead nuclei, and other disintegrating particles. They are sometimes polynuclear; as the cell body does not divide they form also the so-called 'Riesenzellen' of Foot. They are more agile after the first days of incubation. In older cultures they assume round, spherical and oblong shapes, and their enormous protoplasmic body divides up. They then form the cell culture type (fig. 6) the nuclei of which are always vesicular and not very chromatic.

Therefore, in the group of Foot's 'Riesenzellen' do belong besides the products of the involution of the fat cells and the implanted elongated connective tissue celltype with its finely vacuolized plasma, these forms (figs. 22 to 24) in which the nearly fat-less bone marrow of a young chicken was used. This gave conclusive proof that the small mononuclear basophil cell (figs. 8, 35, 36 and 37) after leaving the bone marrow network, can form 'Riesenzellen' which by their nuclear structure resemble connective tissue cells. They later become the cells to which Foot gave the name "cells of the cell culture type." They are enlarged, fat-storing or vacuolized cells capable of phagocytosis.

The results here presented, i.e., the change of the small vesicular basophil cell into true phagocytes and later into 'Riesenzellen' or cells of the cell culture type—were attained by using the bone-marrow of a young, fat-less chicken and the washing out of the undesired cell types, as polymorphonuclear leucocytes. But even if we use the fatty bone-marrow of a full-grown chicken and control the daily changes, the same fact is demonstrated. The first day after incubation (fig. 7) we observe a large number of basophil mononuclear lymphocytes. Three are shown in one microscopic field. Their pale nuclei, often of a lighter blue than the plasma, the irregular shape of their plasmatic body in which sometimes a few fine acidophil granules are

visible, and their large size, make them conspicuous. Examining preparations of the same series a day later, the lymphocytes are very scarce. On the fifth day of incubation, when the disintegrated fat has been disposed of by the phagocytic activity of these basophil cells, characterized by their close position to the network of the bone marrow, they are by far the most numerous types in our tissue cultures. In the following days they grow and divide rapidly forming 'Riesenzellen' which can store fat, become vacuolized, and end in rounding off and becoming cells of the cell culture type, their nuclei with a fine thread-work of chromatin becoming more like true connective tissue nuclei. They can even lose their basophily but may always be distinguished by their nuclear structure from the products of the regressive development of the eosinophil leucocyte in tissue cultures.

It might be possible to interpret Foot's text-figure 5 (page 459, '12) as representing a tissue culture preparation just in such a stage; because the time for formation of these features is the same. But then it is not explained why Foot does not describe the formation of the 'giant cells' and cells of 'cell culture' type after 24 hours' incubation.

In the above mentioned preparations the bulk of all cells, with their fat storing and phagocytic capacities, their vacuolized cytoplasm have now left the implanted bone marrow particle. They advance with their fine, pointed, plasmatic pseudopodia to the outskirts of the plasma clot. Their faintly chromatic nucleus has only two nucleoli. This character is evident in the youngest cells of that kind which are close to the network of the bone marrow (figs. 36 and 37) and is also found in 'Wanderzellen' after Dantschakoff (cf. Dantschakoff, '09, page 133), plate 7, figures 2 to 5. These 'Wanderzellen' which originate from a mesenchyme or endothelial cell can, according to Dantschakoff, either be histiotypic or lymphocytic. They form in the embryonal development specific elements of the connective tissue or the hematopoëtic apparatus, according to the conception of the monophyletic school. In older cultures nearly all basophil cells have nuclei of true connective tissue cell character, i.e., the chromatic granules of the nucleus are connected with fine threads. They are identical with those nuclei figured in figures 22 to 24. Not so frequent are types of nuclei figured in figures 20 and 21.

The 'Wanderzellen' in the tissue culture lose, in the later days of their existence, especially in unrenewed tissue cultures, their fine cytoplasmatic processes but are—by the structure of their nuclei and their cytoplasm—connective tissue cells of a more mesenchymelike character. They are transformed to cells of the cell culture type.

That these cells are descendants of the implanted cells, which were lying close to the bone marrow, is further proved by the following experiment. After all loose cells in the meshes of the bone marrow are washed out by repeated changing of the plasma medium, cells of the type in figures 20 to 24, can be formed. After three changes of the culture medium, with a period of two days between, the cells close to the network formed vacuolized cells which could be interpreted in no other way except as 'Wanderzellen.' Their nuclei had become nearly chromatinless, and their plasma acidophil; they sometimes assumed the character of fat cells, but were generally of the 'Wanderzellen' type.

No large mononuclear lymphocytes could be seen. It is, therefore, also evident that a new formation of this cell type, the mononuclear large lymphocyte of the bone marrow, does not occur in the tissue culture. The smaller and larger basophil cells with a vesicular nucleus near the bone marrow network, and the cells which later leave the network are 'Wanderzellen,' a type closely related to the mesenchymal cell. They can be kept alive for longer periods in renewed culture-medium.

The empty network of the bone marrow, consisting of slender connective tissue cells, has lost its power of sending new cells into the surrounding plasma clot. The network cells remain living for long periods in renewed medium changing only their cytoplasm in the same manner as other connective tissue cells do in plasma culture. It becomes perforated with sieve-like vacuoles which may store fat.

SUMMARY.

The growth of chicken bone marrow in chicken plasma may be divided into two distinct periods. The first period has a more regressive character. As process of this first period may be enumerated:—the degeneration of the erythrocytes and the nearly full-grown

erythroblasts, the ripening of the granulocytes implanted with the bone marrow into the tissue culture; and the decay of the latter.

The eosinophil mononuclear or polymorphonuclear leucocytes after rapid multiplication lose their granules, are flattened out, and form cell chains of acidophil character which undergo slow destruction.

The myelocytes moving at first amoeboid-like in the plasma clot, and behaving like phagocytes, seldom divide, but ripen out until they assume a large size. Then their plasma vacuolizes and disappears, leaving only the nuclei.

The microlymphocytes show no signs of multiplying. They leave the meshes of the bone marrow particle; later lose their cytoplasm; and finally leave their condensed nuclei in the culture.

The large mononuclear lymphocytes of the type occurring in the flowing blood, present in great numbers after the first day of incubation, form now and then fine granules, but undergo no further development into myelocytes. They lose their nuclear chromatin, and their plasma becomes honeycombed and finely vacuolized, and they finally leave as the only trace of their existence faint shadows in the plasma clot.

The so-called fat cells of the bone marrow flatten out; the big fat globules divide into smaller droplets; their plasma either vacuolizes and forms long needle-like projections, or fibroblast-like cells with a central nucleus and honeycombed plasma. The first cell type is phagocytic. These cells represent 'Riesenzellen' in the first period of the tissue culture growth. Not all cells of this type are transformed into fibroblasts or 'Riesenzellen.' Some fat cells disintegrate filling the culture medium with degenerating fat particles. Now and then the nucleus, with a small amount of cytoplasm separates from the dying 'fat cell' and a young 'rejuvenated' cell of fat cell character appears. The so-called fat cells combine the first regressive period of bone marrow growth with the second or more progressive character. Some undergo destruction, some survive, later assuming Foot's cell culture type.

From the first day of incubation, connective tissue cells of elongated shape with very fine pointed projections migrate into the plasma clot. They store fine droplets of fat and partially vacuolize. They are also found in the second period of growth in the tissue culture.

The second period begins with the loosening up of the cells around the network of the bone marrow; the smaller, or larger basophil cells, with vesicular nucleus migrate into the surrounding plasma and the network sends new cells into the plasma clot till it is utterly devoid of cell forms. These cells represent an intermediate type between the 'histiotype Wanderzellen' (Dantschakoff, '09) and the embryonic mesenchyme cell. They do not resemble in all details the large mononuclear lymphocyte of the blood. They move into the surrounding plasma, send out penetrating needle- and bristle-like projections; divide into phagocytes; store fat; lose their projections and partially vacuolize, assuming the form of the "cell culture type."

The network of the bone marrow, having lost its cells, and no longer able to send out emigrating cells, consists of slender connective tissue cells. These show a remarkable paucity of chromatin, are strongly acidophil, and possess sieve-like vacuoles of the finest type.

The 'Riesenzellen' of Foot comprehend several cell types:

1. Transformed fat cells and elongated, vacuolized connective tissue cells.
2. Newly emigrated basophil cells of the bone marrow network, which are related to the "histiotype Wanderzelle" of Dantchakoff.
3. Some few myelocytes and flattened out eosinophil mono- or poly-morphonuclear leucocytes.

These two phenomena, the dying of the cell forms which are not adapted to the continued growth in tissue culture, and the adapting of a new character by those cells which are capable of living longer periods in the plasma medium, often overlaps. They appear more sharply separated in cultures of almost fatless bone marrow, where few 'Riesenzellen' appear in the first days of incubation. From the third to the fifth day, when the loosening of the bone marrow network and its content has begun, they become numerous. The duration of these periods may be stated as follows: The first period lasts from the first to the third day; the second period from the third day to the death of the culture. The surviving cells of the cell culture type (Foot) are modified fat cells and newly formed wandering cells of the mesenchymelike type. After fourteen days' cultivation, they are, except the elongated connective tissue cells the only living cells. They belong to the connective tissue cell type and may, when the medium is renewed, grow indefinitely.

DISCUSSION AND CONCLUSIONS.

As one of the first results of our analytic study, let us discuss the fact that the so-called X or 'Riesenzellen' of Foot represent several different cell types. The myelocytes and larger eosinophil leucocytes acquire, as shown, good dimensions in the tissue culture of bone marrow. The myelocytes, capable of amoeboid moving, form few 'Riesenzellen.' They can easily be omitted in the following discussion, as they are always distinguished by their characteristic nuclei and the blunt form of their projections, when stained and preserved. They are just as unmistakable when living. The large mononuclear or polymorphonuclear eosinophil leucocytes only need be considered, as X or 'Riesenzellen' when they have flattened out and formed rays of cells. Then they are surrounded by the projections of the transformed fat cells or cell types of the 'histiotype Wanderzellen' order. Both cell types are true phagocytes, thus forming, chiefly in the first days after incubation, cell masses of X or 'Riesenzellen' of combined characters. The whole combination may even seem, judged only by its acidophil staining, to be from a different origin. But the daily observations reveal the facts of their development. It is questionable if any necessity exists for giving new names, as Foot did in 1912 and 1913, for the X cells 'Riesenzellen' and later forms. They are either transformed fat cells, or mesenchymelike wandering cells which have left their customary place and which assume in later life in tissue culture the characters of connective tissue.

The name 'Riesenzellen' or true giant cells has already been used for cells of the type represented in figure 10. This multinuclear cell was seen in a tissue culture of bone marrow from a two-months old chicken, and resembles in every particular the true giant cells figured and described by many authors.

To call the questioned basophil cells 'X cells' when their origin is known would be a contradiction. They are either 'fat cells' or mesenchymelike cells, and both types are transformed from their original type by our cultivation method. The present author would propose calling the latter simply wandering mesenchymelike cells, and the fat cells, transformed fat cells. Their close relationship to the mesenchymal cell type is again proved by their physiological behavior in tissue culture, so closely identical with that of the wandering mesen-

chymal type. It even became evident that some 'fat cells' may assume the character of fibroblasts when they are not transformed into highly vacuolized or fat-storing cells of mesenchymal character with projections at first needle-like and later of a rounded or elongated shape. This twofold manner of development of the bone marrow fat cells is important, as it might probably be the result of a non-uniform origin.

In judging the transformations of cell types of mesenchymal origin in tissue culture, we already have established certain facts as a basis of comparison. The mesenchymal cells always grow more rapidly than any other known tissue; they have the ability to store fat; they can vacuolize and can emigrate out of the tissue clot. They can endure this highly artificial method of breeding indefinitely. The bone marrow particle, with its loose meshes, exhibits many 'Wundflächen' which are incited into new growth by the stimulus given by the cutting of the tissue. By repeatedly renewing the culture medium and transplanting the tissue particle, we stimulate the growth again and again, until we have exhausted the power of the network to send newly formed mesenchymelike cells into the plasma, and only a fine thread-like network with a few oblong, small nuclei remains. The pliability of the mesenchymal cell and its ability to undergo transformations is known in embryonic life and is here demonstrated in tissue culture life.

Two subjects of importance have not been touched. Can these wandering mesenchymal cells form fibrils, and have they any relation to the formation of the different elements of the bone marrow? Throughout the whole description of the cell transformations in tissue culture, the writer has avoided Foot's conclusion of 1912, namely, since his X cells form fibrils, they must be of the mesenchymal type. The tissue particle of bone marrow has a fibril-forming connective tissue of its own. When, now and then, fibril-forming cells have been seen (as has been the experience of the writer), they may either originate from cells already implanted in the tissue culture, with the bone-marrow particle, or the imbedded fibrils (Foot '12, plate 22, fig. 15) may represent fibrils or fibers formed by the fibrin-containing plasma of the culture medium (Baitsell '14, '15). Foot maintains that his X cells form fibrils but he does not prove it. Proof could only be

obtained by cultivating isolated cells of a certain known type in a medium which does not contain fibrin as the plasma does. This has never been done and still remains a subject for future investigation.

The author agrees with Foot's view of 1912, that X cells, or the conspicuous cells in tissue cultures of bone marrow, are of mesenchymal type, not because they contain fibrils, but because their origin could be traced and their cytological changes could be recorded. Foot's statement of 1913, must be refuted: that the transformation passed through the stages of small microlymphocyte, macrolymphocytes, large mononuclear forms, myelocytes, polymorphonuclear eosinophil leucocytes, X cells, cell culture type, omitting one or the other forms of this stage, so that directly a lymphocytic origin is considered. It was never observed that true microlymphocytes were transformed into macrolymphocytes in the tissue culture. The basophil cell with vesicular nucleus, pale cytoplasm of various sizes in the network of bone marrow, assumed the cell culture type, after wandering into the cytoplasm, forming point-like projections, displaying the capability of phagocytosis, storing fat, and being vacuolized. There was no stage observed in this transformation which resembled the large mononuclear lymphocyte or the 'lymphocytoid Wanderzelle' of Dantschakoff, though this type could be easily observed in chicken bone marrow when the bird had cyanolophia. The close resemblance with Dantschakoff's 'histiotype Wanderzelle'—cells which form ('09, page 177), after some changes, the 'ruhenden' wandering cells of the connective tissue—could only be discovered when the basophil forms left the net-work and began to emigrate.

It appears highly plausible that in tissue culture the indifferent mesenchymelike cell in the bone marrow net work does not show its supposed duality, either to form the known elements of the connective tissue or according to the views of the monophyletic school, the different elements of the hemato- and granulopoësis. In a medium, where circulation has ceased, where no oxygen renovation takes place, the potency to form the lymphocytic elements of bone marrow may not be strong enough to overcome the potency to form fat cells, fibroblast and 'histiotype' wandering cells. Therefore the present series of experiments does not prove anything concerning the views of the mono- or duophyletic schools, of the formation of blood and

lymph in the bone marrow. Here renewed experiments should be made, the different cell types after emigration should be isolated and submitted to conditions reproducing either the condition of the blood or of the lymph. Only with still more refined methods would it seem possible to elucidate, outside the body, the complicated process of blood and lymph formation.

But this series of experiments proves that the latent qualities of the basophil mononuclear cells in the meshes of the bone marrow can arise *de novo* in the adult animal, because their wandering phagocytic, fat-storing character has been made evident. This fact ought to be considered in dealing with the appearance of these, and related cell types in the blood and lymph during diseases.

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EXPLANATION OF PLATES.

The drawings were made from total preparations, with Abbe camera lucida, Zeiss homogeneous immersion 2 mm. and compensating ocular 12, with drawing board level with stage of microscope. Magnification about 1500 diameters.

PLATE 1.

EXPLANATION OF FIGURES.

The bone marrow used for the preparations shown in figures 1 to 6 was taken from a well fed, full grown chicken containing a large amount of fat. It was incubated at a temperature of 38°C. in the chicken plasma medium.

1 The first cells emigrating from the particle into the plasma. Bone marrow one hour in plasma, January 3, 1916, 10 a.m. to 11 a.m. Two mononuclear eosinophil leucocytes, one lymphocyte, and one normoblast are visible.

2 Cells which have left the implanted bone marrow particle after twenty-four hours and emigrated into the plasma. January 3 to January 4, 1916. Mononuclear and polynuclear eosinophil leucocytes with rod-shaped granules and large granulocytes with rounded, highly refractile granules are visible. Two fat cells at the right and left side of the preparation have divided up their big fat globule into small fat droplets (compare plate 6). In the middle a large non-granular lymphocyte is to be seen.

3 Cells which have left the implanted bone marrow particle and have advanced to the border of the plasma clot after forty-eight hours' incubation. January 3 to January 6, 1916. One large 'Riesenzelle' and a small granulocyte with highly refractile granules are visible together with one small lymphocyte with vesicular nucleus. Red blood corpuscles with or without nuclei are present. One red blood corpuscle extrudes its nucleus.

6 Cells which have stayed two hundred and sixteen hours in the plasma medium December 25, 1915 to January 3, 1916. Cell culture types.

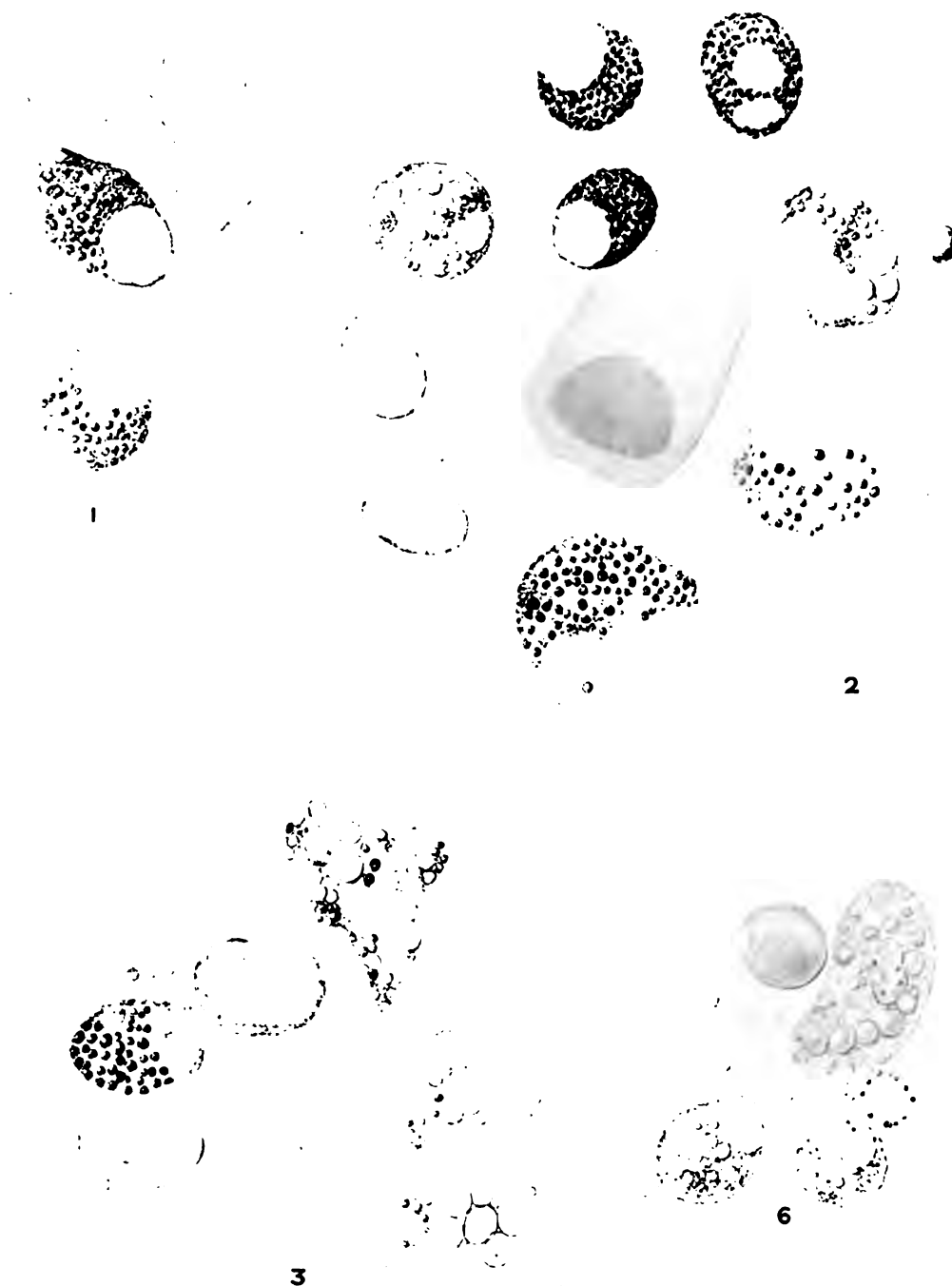
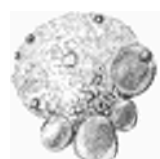
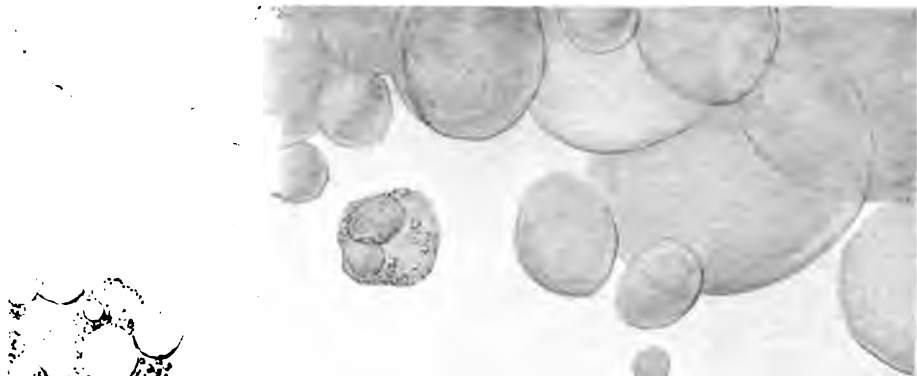


PLATE 2.

EXPLANATION OF FIGURES.

4 Cells near the implanted tissue particle after seventy-two hours' incubation. January 3 to January 6, 1916. Extrusion of fat droplets and breaking up of the 'Riesenzellen.'

5 Cells on the outskirts of the surrounding plasma after ninety-six hours' incubation. Disintegration of fat cells. Note the very small leucocyte.



4



5

PLATE 3.

EXPLANATION OF FIGURES.

7 Total preparation: Bone marrow of a full grown, well fed chicken after thirty hours' incubation at 38°C. in the plasma medium. January 3 to January 4, 1916. Orth's fluid, Giemsa stain. (Compare for explanation pages 616 and 624-626). Actual field represented.

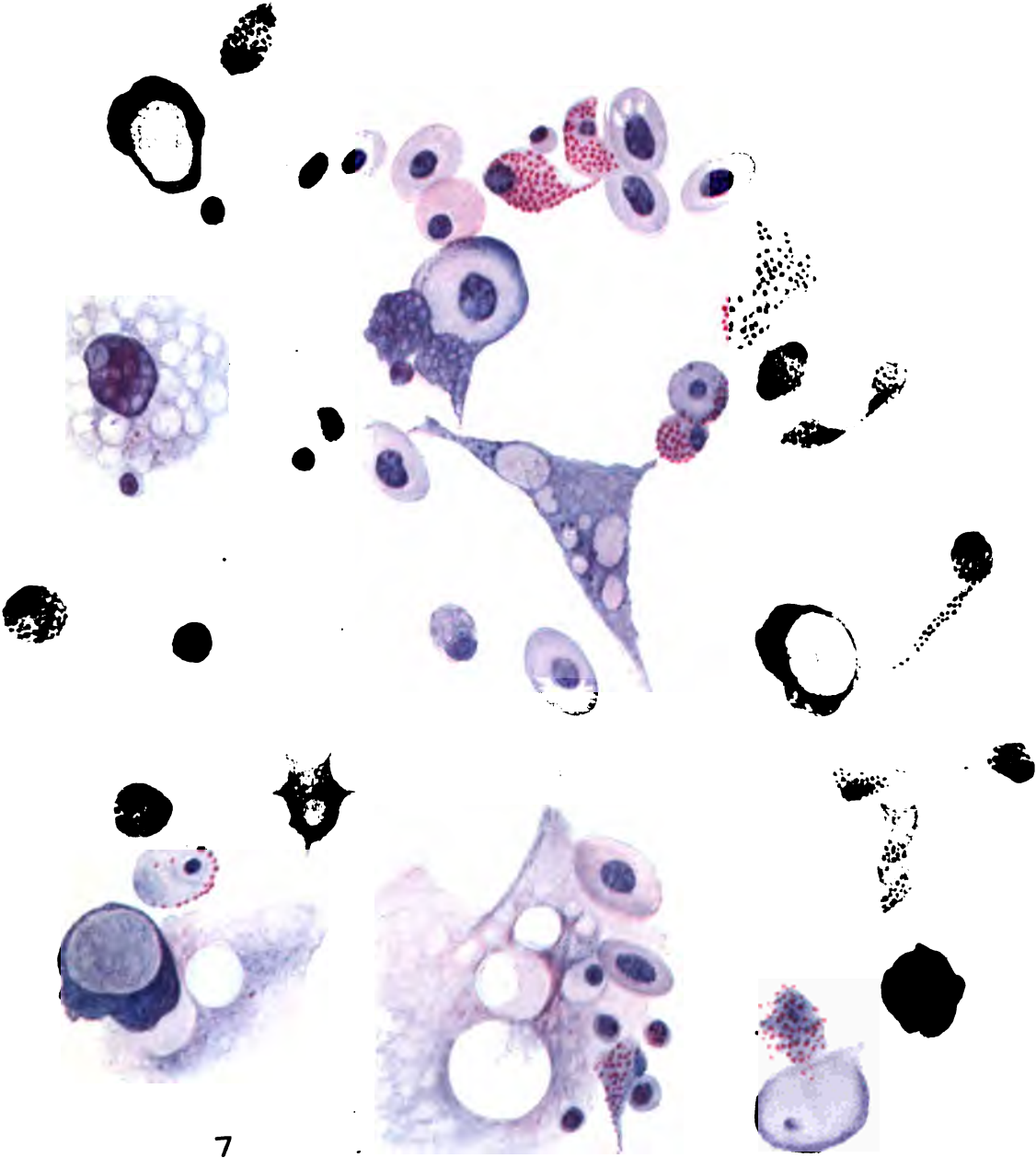
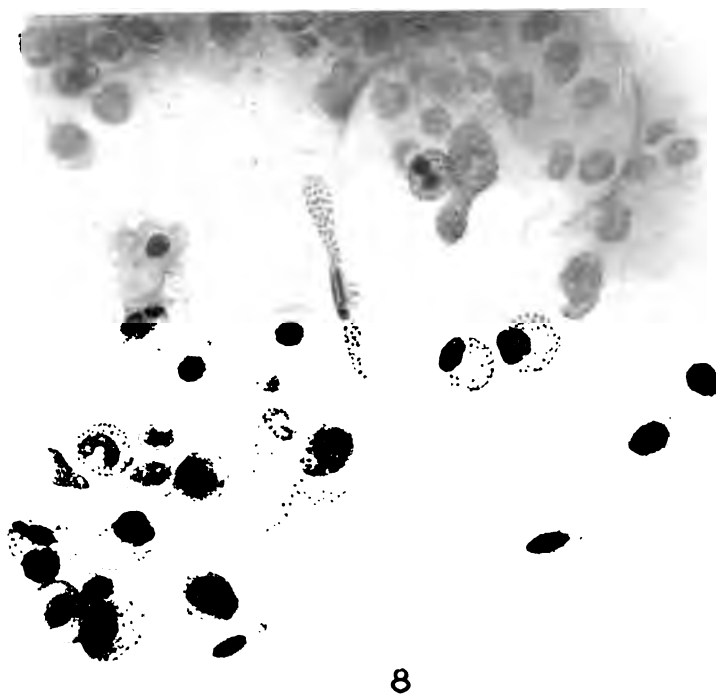


PLATE 4.

EXPLANATION OF FIGURES.

8 Total preparation: Bone marrow of a chicken not yet full grown, with a small amount of fat, after ninety minutes' incubation at 38°C. in the plasma medium. June 7, 1916. Orth's fluid, Giemsa stain. Small eosinophil leucocytes and many basophil cells with vesicular nuclei are present.

9 Total preparation: Bone marrow of a full grown, well fed chicken, after twenty-four hours' incubation at 38°C. in the plasma medium. December 14 to December 15. Orth's fluid, hematoxylin, eosin stain. The slender vacuolized cell with its nucleus of connective tissue cell structure is already visible after this short incubation period.



8



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PLATE 5.

EXPLANATION OF FIGURES.

10 Giant cell from the bone marrow of a young, but full grown, well fed chicken, after one day's incubation; to represent the type which is generally named giant cell and is not identical with Foot's 'Riesenzelle.'

11 to 19 White bone marrow of a young, nearly fatless chicken in tissue culture at 38°C. After one hour's incubation the tissue particle was extracted and the emigrated cells were allowed to develop further. February 11 to February 25, 1916. A detailed description of the changes of the eosinophil leucocytes is given on pages 609-611.

20 to 27 The same bone marrow particle after having been freed from its eosinophil leucocytes by the above described process was implanted for one day again in a plasma medium and extracted again. The emigrated cells were allowed to develop from February 12 to February 25, 1916. Figures 20 and 21 represent a cell type more related to fat cells, figures 22 to 24 a type more related to connective tissue cells, figures 25 to 27 show known cell types which have not changed their character in the tissue culture. Note figure 24: a so-called form of the cell culture type. All cells on plate 5 are conserved in Orth's fluid and stained with Giemsa stain.

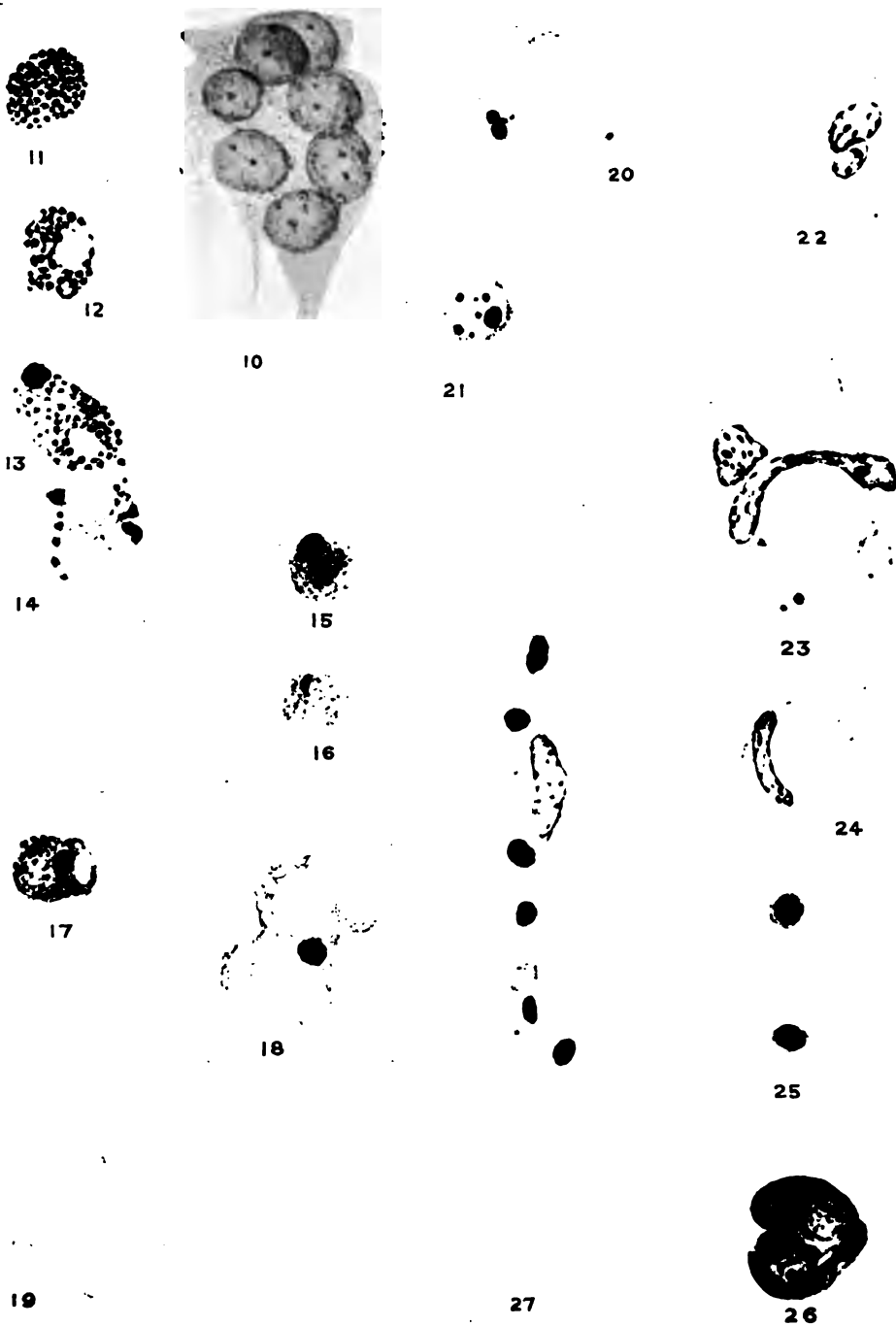
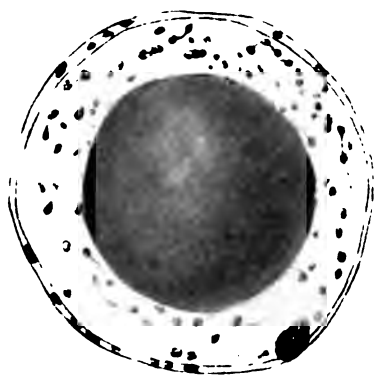


PLATE 6.

EXPLANATION OF FIGURES.

28 to 32 Involution of the so-called fat cells of the bone marrow to 'Riesenzellen' in the plasma medium. White bone marrow of a younger well fed, full grown chicken in tissue culture from November 30 to December 1, 1915. Conservation: Formol. Osmium, Safranin stain.



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PLATE 7.

EXPLANATION OF FIGURES.

33 and 34 White bone marrow from a younger full grown, well fed chicken in tissue culture at 38°C. from February 29 to March 1, 1916. Foot's 'Riesenzellen' already present after one day's incubation.

35, 36 and 37 An identical piece of bone marrow, as described above, was extracted after three hours and transplanted in a new culture medium. The next morning, again extracted and transferred in a new medium. After eight hours the preparation was conserved and the cells nearest to the network studied. A cell, 35, of this preparation having migrated from the network, showing phagocytosis.

38 A cell of the network which begins to become disconnected.

39 to 42 White bone marrow from a full grown chicken in tissue culture from February 22 to March 2, 1916, at 38°C. Regressive changes of cells of the large mononuclear type or the myeolocytic type.

The cells figured on figures 33 to 38 were conserved with Orth's fluid, those on figures 38 to 42 with Schaudinn Sublimat Alcohol and stained with Giemsa stain.



33



34



35



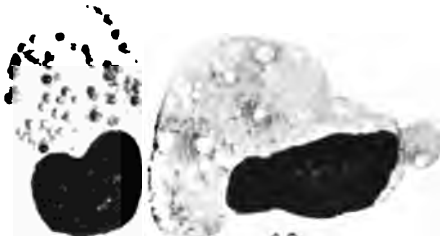
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PLATE 8.

EXPLANATION OF FIGURES.

43 Emigrated cells after three hours' incubation (mono- and poly-nuclear eosinophil leucocytes).

44 Emigrated cells after 24 hours' incubation (mononuclear basophil cells) after the culture medium has been once changed after three hours.

Compare pages 620-626 for detailed description.

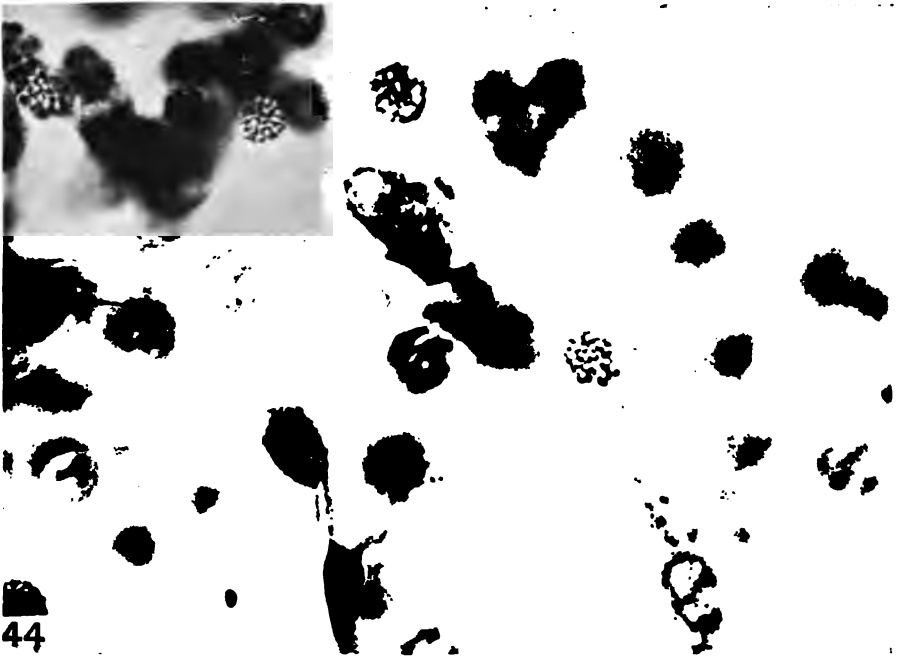
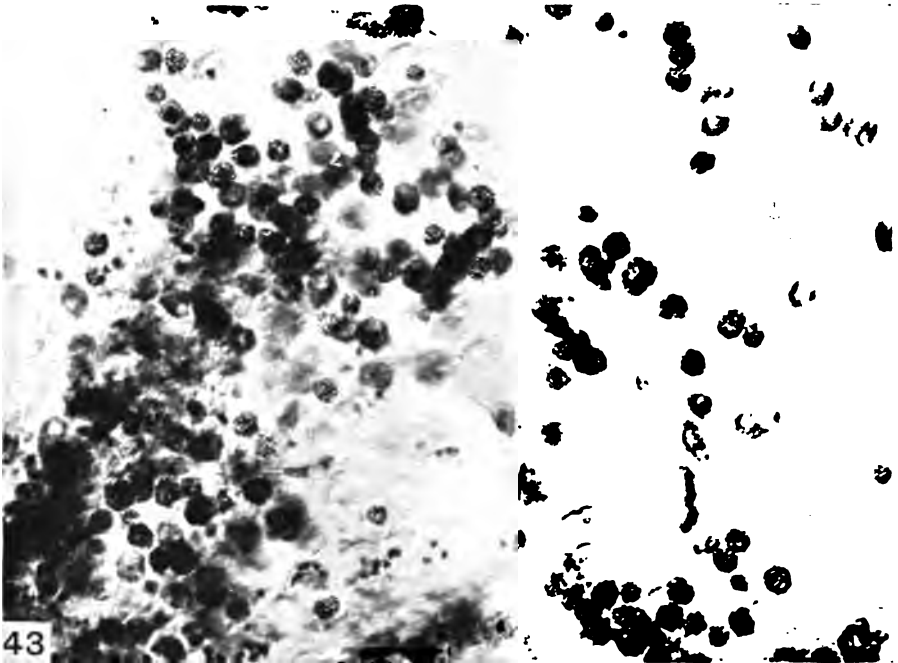
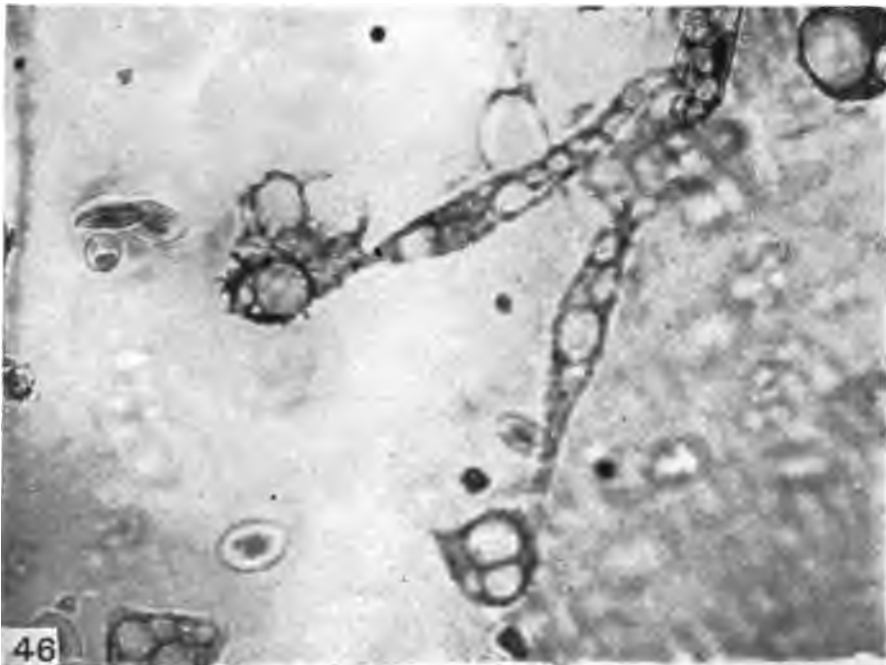


PLATE 9.

EXPLANATION OF FIGURES.

45 Involution of the fat cells after 24 hours' incubation near to the implanted bone marrow particle.

46 Involution of the fat cells after 24 hours' incubation. Cells near the periphery of the plasma clot. Compare figures 33 and 34, plate 7, from the same series of experiments.



INCREASED VIRULENCE OF THE HOG-CHOLERA BACILLUS PRODUCED BY PASSAGE THROUGH RABBITS.

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(Received for publication, May 18, 1917.)

In a previous paper (1) the writer mentioned the attempt to modify the carbohydrate reactions of a culture of the hog-cholera bacillus by passing it through a series of rabbits. The only change noted was an increase in virulence, and it is the object of this paper to record this change.

Since the time of Pasteur many organisms have had their virulence increased by animal passages but the recorded results of the passage of the hog-cholera bacillus through rabbits are somewhat conflicting. Moore (2) passed a typical organism through a series of twenty-six rabbits and concluded that there was no increase in virulence for this animal. He judged the virulence by the time taken to kill the animal and did not consider the amount of culture necessary to produce death. Previous to this Selander (3) had reported the rapid increase in virulence of a hog-cholera bacillus by rabbit passages and his work was apparently confirmed by Metchnikoff (4). Smith and Moore (5) had shown, however, that Metchnikoff was working with the swine-plague bacillus and as Moore could not confirm the work of Selander he concluded that the latter was probably also working with the swine-plague organism.

Smith (6) later worked with a culture of the hog-cholera bacillus of low initial virulence further attenuated by age, which would not kill rabbits when injected under the skin. By animal passages, he increased the virulence to the point where subcutaneous injections were fatal.

Later Smith and Reagh (7) in attempting to modify the agglutinability of a strain of the hog-cholera bacillus, passed it through a series of fourteen rabbits. As a result of this passage the virulence was increased from a minimal fatal dose of 0.1 cc. in the stock strain to 0.00001 cc. in the passage strain. It has already been pointed out (1) that this increased virulence still persists to a certain degree after a lapse of 15 years.

The organism with which we worked is a stock culture known as Hog-cholera XII. It was isolated by Dr. Smith in 1914 from the spleen of a pig dying from hog-cholera. Soon after this it was passed through a rabbit and since that time it has been kept on slant agar in the cold, transfers being made monthly.

Culturally it is a motile, Gram-negative rod, growing readily on the ordinary media and forming acid and gas in dextrose bouillon but not attacking lactose or saccharose. It is quantitatively agglutinated by serum from rabbits injected with other strains of the hog-cholera bacillus and when injected into animals it causes the production of agglutinins for other strains of the hog-cholera bacillus.

Starting in January 1916 this culture was passed through a series of eleven rabbits; the essential details of this passage will be found in Table I. The passage was made directly from one animal to the next by using a suspension of crushed spleen for the inoculation. After the third transfer the inoculation was made by rubbing a small amount of the spleen suspension into the shaven skin of the next rabbit.

TABLE I.
Passage of Hog-Cholera XII through Rabbits.

Passage.	Weight of rabbit.	Method of infection.	Material and dose.	Length of life.	Remarks.
				days	
1	1,199	Subcutaneous.	0.5 cc. of 24 hr. bouillon culture.	6	Typical lesions.
2	1,172	"	Spleen suspension.	6	" "
3	1,274	"	" "	7	" "
4	1,305	Cutaneous.	" "	7	" "
5	1,430	"	" "	7	" "
6	2,073	"	" "	9	" "
7	2,089	"	" "	10	" "
8	2,273	"	" "	8	" "
9	2,029	"	" "	7	" "
10	2,308	Subcutaneous.	0.000001 cc. of 24 hr. bouillon culture from Rabbit 9.	9	" "
11	2,336	"	0.00000001 cc. of 24 hr. bouillon culture from Rabbit 10.	6	" "

It would be difficult to estimate accurately the relative virulence of this culture from the duration of the disease in the various animals but a comparison of the number of organisms necessary to kill gives us very definite results. With the stock culture one must use somewhat over 0.00001 cc. of a 24 hour bouillon culture to produce death.

Of four rabbits given a subcutaneous injection of this amount one died in 12 days, one showed an increased temperature and loss in weight, the other two showed no effects. One rabbit that was given 0.001 cc. subcutaneously died in 4 days. With the strain of the same bacillus passed through the rabbits, 0.00000001 cc. of a 24 hour bouillon culture injected subcutaneously into a rabbit weighing 2 kilos causes death in about 6 days. The passage strain has therefore been increased in virulence about one thousand times and plate counts show that the number of organisms necessary to infect has been reduced from approximately 20,000 to 20.

The type of disease produced by the more virulent organism shows no striking departure from that caused by the original culture. This organism seems to have a greater power of penetration than do most cultures of the hog-cholera bacillus, as it causes only a slight local lesion. The bacteria apparently enter the body through the lymphatics, for the axillary and inguinal lymph nodes on the side of the inoculation are enlarged and congested and often show large areas of necrosis.

The passage through rabbits has produced no change in the morphology of the organism that can be detected either in films made from the spleen of an animal dying from infection or in films made from cultures. The passage strain is slightly more motile than the original culture but this difference is not marked. It is also more susceptible to agglutinins, in that clumps are formed in a shorter time, but the readings after 2 hours' incubation are the same. The passage of the original strain through one rabbit makes it as susceptible to agglutinins as is the strain passed through eleven rabbits. With some immune sera the parent strain is agglutinated in slightly higher dilutions than is the passage strain but here too the difference is not great.

SUMMARY AND CONCLUSION.

By passage through a series of eleven rabbits a culture of the hog-cholera bacillus has increased its virulence a thousand times. A subcutaneous injection of twenty organisms, or 0.00000001 cc. of a 24 hour bouillon culture, or a drop of a bouillon culture rubbed lightly into the shaven skin, produces, in the rabbit, a characteristic disease resulting in death on or about the 6th day.

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THE SIGNIFICANCE OF AGGLUTININS IN THE IMMUNITY OF THE RABBIT TO THE HOG-CHOLERA BACILLUS.

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(Received for publication, May 18, 1917.)

A great deal of experimental work has been done on agglutinins, yet we have very little evidence as to the part they play in immunity to any given infectious disease. Much of the work tends to show that they are not indicators of immunity but in spite of this the agglutinin titer is often used as an index of immunity after antityphoid vaccination and when the titer falls it is regarded as an indication for revaccination. In the production of various immune sera it is often assumed that the height of the agglutination titer is an index of the antibody content but the evidence on which this assumption is based is not clear. We have endeavored to throw some light on the relation of agglutinins to immunity by the use of the highly virulent culture of the hog-cholera bacillus described in the previous paper (1).

A large part of the experimental work on agglutinins has been done with the rabbit and the horse inoculated with the typhoid bacillus, and the great weakness of this work is that in the former animal at least, this organism does not produce a true invasive disease but acts only when large numbers of bacteria are given. On the other hand, the hog-cholera bacillus produces, in the rabbit, a true disease resembling in many respects typhoid fever in man.

HISTORICAL.

In 1894 Smith and Moore (2) showed that heated cultures of the hog-cholera bacillus would not produce an immunity in the rabbit but that the injection of a living attenuated culture would produce an active immunity to the more virulent organism. They did not at this time study the agglutinins, but later Smith and Reagh (3) produced agglutinins by the injection of heated as well as living cultures of the hog-cholera bacillus.

Shoukévitch (4) reports work similar to that to follow in which, using the hog-cholera bacillus and the rabbit, he showed that the injection of heated cultures caused an increase in the complement-fixing bodies, agglutinins, and opsonins but that this was not associated with an immunity. The injection of a living culture of low virulence caused a very slight production of these bodies but did cause an active immunity to more virulent organisms. Of fifteen animals tested in this way, five showed no effects, one died with a typical septicemia, two died from acute intoxication, and seven died in from 15 to 70 days with a paralysis of various groups of muscles. All showed little or no increase in the immune bodies following the injection of the culture of low virulence.

Whether the phenomenon described by Bull (5) under the name of *intra vitam* agglutination is related to *in vitro* agglutination is a question that will not be considered in this paper but we will consider the relation of this phenomenon to immunity to the hog-cholera bacillus. Bull has recorded experiments with a rather large series of organisms, the avirulent members of which are clumped when injected intravenously into normal rabbits, while the virulent members are not clumped and are found in the circulation for some time after the injection. When the bacilli are clumped they rapidly disappear from the blood stream and in a half hour after the injection, films from the liver and lungs show many polymorphonuclear leukocytes filled with organisms. Virulent organisms are not clumped in the circulation of normal rabbits but are rapidly clumped and phagocytized when injected into immune animals. Bull says (5) "The degree of agglutination and opsonization of bacteria within the animal body is inversely parallel to the infectiousness of the bacteria for the host" but he is careful to state that exceptions may be found to this rule.

EXPERIMENTAL.

Cultures Used.—Reference has already been made to the highly virulent test culture (1). The parent culture that had not been passed through rabbits and having the laboratory number XII was used as a vaccine and in small doses of the living culture to produce an immunity.

Hog-cholera Neb., isolated by Dr. Smith in 1886 (6), is now a culture of very low virulence. It is a typical hog-cholera bacillus except for its lessened virulence.

Hog-cholera Ark., isolated by Dr. Dinwiddie in 1889, was referred to by Dr. Smith in 1903 (3) and the cultural characters have been reported more recently (7). It is an organism of moderate virulence, probably due in part at least to its prolonged cultivation on artificial media.

Use of Heated Cultures.

The first experiment was made with a vaccine prepared by suspending the 24 hour growth from an agar slant inoculated with Hog-cholera XII in 10 cc. of salt solution and heating for 1 hour at 60°C. The heated suspension was incubated over night and several loops were transferred to bouillon in order to be sure that it was sterile. Having proven that all the bacteria had been killed the vaccine was injected subcutaneously into two rabbits of approximately equal weight. The first injection was of 0.5 cc. and was followed in 5 days by 1 cc. 6 days later they were each given 2 cc. Both rabbits bore the injections well, showing only a slight and temporary loss in weight.

18 days after the beginning of the treatment and 7 days after the last inoculation these two rabbits, together with a control, were bled from the ear vein and the serum of the inoculated animals was tested for agglutinins against the strain of the hog-cholera bacillus with which they had been injected and also against the strain that had been passed serially through rabbits and with which they were to be inoculated in order to test their immunity.

In order that the rabbits might have every opportunity to show any immunity they might have gained by the injection of the heated cultures they were tested by the cutaneous method, two drops of a 24 hour bouillon culture being lightly rubbed into the shaven skin of each animal. This test was made 2 days after the bleeding from the ear vein and 9 days after the last injection of the heated cultures. The results of this experiment are summarized in Table I.

TABLE I.

Test of the Power of Heated Cultures to Produce Agglutinins and Immunity.

Rabbit No.	Vaccinated.	Limit of agglutination* for Hog-cholera XII.		Weight.	Immunity test. Result of cutaneous inoculation with test culture
		Stock.	Passage strain.		
1	Three times with heated culture.	$\frac{1}{12,800}$	$\frac{1}{12,800}$	1,823 gm.	Died in 5 days.
2	Same as No. 1.	$\frac{1}{12,800}$	$\frac{1}{12,800}$	1,889	" " 7 "
3	Control.	Not tested.		2,006	" " 6 "

* The limit of agglutination is the highest dilution in which clumps of bacteria can be seen with the naked eye. A 24 hour bouillon culture was used as an antigen throughout.

It will be seen that while both treated rabbits had in their serum agglutinins that could be demonstrated in a dilution of $\frac{1}{12,800}$ they

had no immunity. Both died in approximately the same number of days after the inoculation as did the control. At autopsy all three rabbits showed the same local as well as visceral lesions, the only essential difference being that both vaccinated rabbits had a small area of pneumonia. It is quite possible, however, that the latter was due to another organism that was affecting our stock rabbits.

A similar experiment, but with results that are not so clear-cut, was made some time later. The preparation of the vaccine and the dosage was the same as in the preceding experiment except that the culture used was the highly virulent rabbit passage strain. The test for immunity was made by injecting subcutaneously 0.0000001 cc. of a 24 hour bouillon culture of the passage strain. The results are summarized in Table II and show that of the vaccinated rabbits the one that had the higher agglutination titer died, while the other one survived without showing any marked effects from the inoculation. Examination of the controls shows that we were using about the smallest dose that would cause an infection, so that the apparent immunity of the one vaccinated rabbit might be due to causes other than the injected vaccine such as a natural immunity or too small an infecting dose. In spite of this discordant result it is clear that there was no demonstrable immunity in the rabbit with the higher agglutination titer.

TABLE II.

Test of the Power of Heated Cultures to Produce Agglutinins and Immunity.

Rabbit No.	Vaccinated.	Limit of agglutination for test culture.	Weight.	Subcutaneous injection of test culture.	
				Amount.	Result.
4	Three times with heated culture.	$\frac{1}{25,600}$	2,011 gm.	0.0000001 cc.	Died in 9 days.
5	Same as No. 4.	$\frac{1}{12,800}$	2,030	0.0000001	No effect.
6	Control.	0	2,683	0.0000001	Died in 9 days.
7	"	0	1,763	0.00000001	No effect.

Use of Living Cultures to Produce Immunity.

The immunization of rabbits by the injection of living cultures of the hog-cholera bacillus is very difficult, for their resistance may be overcome by the injection of too large doses or they may succumb to

some spontaneous disease to which they seem very susceptible during such treatment. We did succeed in getting four animals to the stage where they could be tested against the highly virulent culture and as the treatment of each animal is different from the others a summary of the immunization is given below.

Rabbit 8.—Three injections of a non-virulent strain followed by a mildly virulent strain.

June 6, 1916. Subcutaneous injection of 0.01 cc. of a 24 hour bouillon culture of Hog-cholera Neb. Slight rise in temperature but no loss in weight.

June 20. Subcutaneous injection of 1 cc. of a 24 hour bouillon culture of Hog-cholera Neb. Loss in weight but no increase in temperature.

July 8. Intravenous injection of 0.1 cc. of a 24 hour bouillon culture of Hog-cholera Neb. Rise in temperature but no loss in weight. Another rabbit that had had the same injections died during the night following the intravenous inoculation.

July 21. Subcutaneous injection of 0.1 cc. of a 24 hour bouillon culture of Hog-cholera Ark. No loss in weight and no rise in temperature. Previous tests had shown that this culture in this amount would kill a normal animal in 20 days.

This rabbit was bled three times after the last inoculation and the results of the agglutination tests are given in Table III.

TABLE III.

Agglutination Titer of the Serum of Rabbit 8.

Culture agglutinated.	Limit of agglutination for serum drawn on.		
	Aug. 4	Sept. 19	Oct. 3
Hog-cholera XII	$\frac{1}{80,000}$	$\frac{1}{51,200}$	$\frac{1}{3,200}$
“ XII, passage series	$\frac{1}{20,000}$	$\frac{1}{25,600}$	$\frac{1}{6,400}$

Rabbit 9.—Injected with increasing numbers of Hog-cholera Ark. (mildly virulent strain).

June 6, 1916. Subcutaneous injection of 0.0001 cc. of a 24 hour bouillon culture. Rise in temperature but no loss in weight.

June 20. Subcutaneous injection of 0.01 cc. of a 24 hour bouillon culture. Rise in temperature and loss in weight extending over a period of 20 days.

July 21. Subcutaneous injection of 0.1 cc. of a 24 hour bouillon culture. Slight loss in weight but no rise in temperature.

This last injection was one that would kill the normal rabbit, so no further injections were given but the animal was allowed to rest until it was tested for immunity to the highly virulent strain. During this period of rest it was bled three times and the limit of agglutination for the hog-cholera bacillus determined. The results of these tests are given in Table IV.

TABLE IV.
Agglutination Titer of the Serum of Rabbit 9.

Culture agglutinated.	Limit of agglutination for serum drawn on.		
	Aug. 4	Sept. 19	Oct. 3
Hog-cholera XII	$\frac{1}{20,000}$	$\frac{1}{3,200}$	$\frac{1}{1,600}$
“ XII, passage series	$\frac{1}{5,000}$	$\frac{1}{1,600}$	$\frac{1}{1,600}$

Rabbit 10.—Injected with increasing amounts of Hog-cholera XII (virulent culture).

June 20, 1916. Subcutaneous injection of 0.0000001 cc. of a 24 hour bouillon culture. Rise in temperature but no loss in weight.

July 11. Injection of June 20 repeated. No rise in temperature and no loss of weight.

July 21. Subcutaneous injection of 0.00001 cc. of a 24 hour bouillon culture. No loss in weight or rise in temperature.

Aug. 7. Subcutaneous injection of 0.001 cc. of a 24 hour bouillon culture. No effect on weight or temperature. Control rabbit died in 4 days

Aug. 19. Subcutaneous injection of 0.01 cc. of a 24 hour bouillon culture. No effect on weight or temperature. The animal was now allowed to rest for some time before its resistance to the highly virulent strain was tested. Table V gives the results of the agglutination tests made during this period.

TABLE V.
Agglutination Titer of the Serum of Rabbit 10.

Culture agglutinated.	Limit of agglutination for serum drawn on.		
	Aug. 31	Sept. 17	Oct. 3
Hog-cholera XII	$\frac{1}{25,600}$	$\frac{1}{25,600}$	$\frac{1}{6,400}$
“ XII, passage series	$\frac{1}{25,600}$	$\frac{1}{25,600}$	$\frac{1}{6,400}$

Rabbit 11.—One injection of a sublethal number of Hog-cholera XII bacilli (virulent culture).

Aug. 22, 1916. Subcutaneous injection of 0.00001 cc. of a 24 hour bouillon culture of Hog-cholera XII. Marked loss in weight and rise in temperature. Another rabbit inoculated with the same amount of culture at the same time died in 12 days. In Table VI are given the results of the agglutination tests made on the serum of this rabbit previous to its inoculation with the highly virulent culture.

TABLE VI.
Agglutination Titer of the Serum of Rabbit 11.

Culture agglutinated.	Limit of agglutination for serum drawn on.	
	Sept. 18	Oct. 3
Hog-cholera XII	$\frac{1}{6,400}$	$\frac{1}{3,200}$
“ XII, passage series	$\frac{1}{3,200}$	$\frac{1}{3,200}$

On Oct. 3 these four rabbits together with two controls were bled from the ear vein and the sera tested for agglutinins to the highly virulent culture. 3 days later each was given a subcutaneous injection of 0.000001 cc. of a 24 hour bouillon culture of the highly virulent organism in order to test their immunity. The necessary data for the understanding of this test together with the results are given in Table VII. The subcutaneous route was chosen for the inoculation in order to be sure that all the animals received the same amount of culture.

Examination of the table will show that the rabbits previously injected with living cultures were not affected when inoculated with the highly virulent culture, whereas the controls died in 7 days. The agglutination titer of the sera of each of these immune animals was below that found in the serum of the animals injected with heated cultures, yet the latter promptly succumbed to an inoculation with this virulent culture in small amounts.

3 months later two of these immune rabbits were again tested for agglutinins and then injected with a large amount of the highly virulent culture. The results are summarized in Table VIII where it will be seen that one rabbit did not survive this severe test while the animal with the lower titer resisted one thousand times the minimal fatal dose.

TABLE VII.

Test of the Power of Living Cultures to Produce Agglutinins and Immunity.

Rabbit No.	Immunization.				Test of immunity to Hog-cholera XII, passage series, on Oct. 6, 1916.			
	Date of injection.	Strain of hog-cholera.	Amount of 24 hr. bouillon culture injected.	Route.	Time after last injection.	Weight.	Agglutination titer 3 days before test of immunity.	Result of subcutaneous injection of 0.000001 cc. of 24 hr. bouillon culture.
8	1916		cc.		days	gm.		
	June 6	Neb.	0.01	Subcutaneous.	77	3,028	$\frac{1}{6,400}$	No effect.
	" 20	"	1.0	"				
	July 8	"	0.1	Intravenous.				
9	" 21	Ark.	0.1	Subcutaneous.				
	June 6	Ark.	0.0001	Subcutaneous.	77	3,374	$\frac{1}{1,600}$	No effect.
	" 20	"	0.01	"				
	July 21	"	0.1	"				
10	June 20	XII	0.0000001	Subcutaneous.	48	2,768	$\frac{1}{6,400}$	No effect.
	July 11	XII	0.0000001	"				
	" 21	XII	0.00001	"				
	Aug. 7	XII	0.001	"				
	" 19	XII	0.01	"				
11	Aug. 22	XII	0.00001	Subcutaneous.	45	2,024	$\frac{1}{3,200}$	No effect.
12	Normal animal for control.				0	2,932	0	Died in 7 days. Typical lesions.
13	Normal animal for control; injection one-tenth the amount given other rabbits.				0	2,359	0	Died in 7 days. Typical lesions.

The results of these tests clearly indicate that an animal may show agglutinins to the hog-cholera bacillus *in vitro* and yet have no immunity. It cannot be said, however, that these bodies have no relation to immunity for they are present in the sera of all the immune animals though, at the time of the test, not in as high dilutions as in the vaccinated rabbits.

TABLE VIII.

Test of the Power of Living Cultures to Produce Agglutinins and Immunity.

Rabbit No.	Immunisation.	Agglutination titer 3 days before test of immunity.	Test of immunity.	
			Amount of 24 hr. bouillon culture injected.	Result.
8	See Table VII.	$\frac{1}{1,280}$	0.0001	Died in 8 days.
11	" " VII.	$\frac{1}{640}$	0.0001	Slight rise in temperature.
6	Control.	0	0.0000001	Died in 9 days.
7	"	0	0.0000001	No effects.

Attempts have been made to differentiate after the method of Joos (8) the agglutinins in the vaccinated rabbit from those found in the immune animals. No difference has been found in the susceptibility to heat of the agglutinins from these two sets of animals nor do the sera act differently on heated bacteria.

Intra Vitam Agglutination.

The work of Bull (5) suggested the comparison of the *intra vitam* agglutination in vaccinated rabbits and in those immunized by the use of living cultures. When a suspension of the living organisms from the highly virulent strain are injected intravenously into either of these animals they are promptly clumped and rapidly disappear from the circulation. Films made from the liver half an hour after the injection show cells packed with bacteria. Most of the phagocytic cells found were polymorphonuclear leukocytes but a few phagocytic endothelial cells were also present. No difference was noted in the reactions of animals from the two sets though one was immune and the other was very susceptible to the bacterium injected. The most interesting phase of this work was that the control animals also showed typical *intra vitam* clumping. This fact was verified repeatedly but only one experiment will be given.

The growth from two 24 hour agar slants inoculated with Hog-cholera XII passage virus was suspended in 15 cc. of salt solution. Shaken and centrifugal-

ized for $\frac{1}{2}$ hour. Removed the supernatant fluid and suspended the residue in 5 cc. of salt solution and shook vigorously to break up clumps. Injected intravenously into a normal rabbit weighing 2,270 gm. Blood removed from the heart at stated intervals and dilutions made for plate cultures. The first dilution was made in a glass-stoppered bottle and was shaken for some time to break up clumps. At the same time films were made that later were stained with Manson's methylene blue. The findings in the films as well as the results of the plate counts are given in Table IX.

40 minutes after the injection, the rabbit was chloroformed and films were made from the liver. Cells containing bacteria were present but were not numerous.

TABLE IX.

Intra Vitam Agglutination Test Using a Normal Rabbit.

Time after injection.		Bacteria per cc. in heart's blood.	Result of examination of film.
min.	sec.		
0	31	30,000,000	Large clumps of bacteria embedded in a blue-staining homogeneous mass.
2	30	4,800,000	No bacteria found.
5	24	5,950,000	" " "
15	0	75,000	" " "
30	0	117,000	" " "

This experiment, as well as the others made, shows that in the normal rabbit there is a prompt clumping of the injected bacteria, a rapid disappearance from the blood stream as shown by films and plate counts, and a phagocytosis of the bacteria by cells in the liver and in other organs. The centrifugalization of the bacteria had nothing to do with the clumping for it occurred in other animals where a suspension made directly from the agar slant was injected. When the dilutions of blood were not shaken in glass-stoppered bottles, plates made 30 minutes after the injection and containing $\frac{1}{500}$ cc. of blood were sterile.

SUMMARY AND CONCLUSION.

Rabbits may show a high agglutination titer to the hog-cholera bacillus and have no immunity and on the other hand immune animals may have a comparatively low agglutination titer. In other words, with this organism the height of the agglutination titer does

not indicate the degree of immunity. As this bacillus so closely resembles the typhoid bacillus biologically and pathologically, it seems safe to conclude, until evidence is brought forth to the contrary, that in man the height of the agglutination titer does not indicate the actual degree of immunity to the latter organism. The same would apply to other members of the typhoid-colon group. It would not be wise to draw a more general conclusion until other organisms have been tested. This does not mean that agglutinins are not related to immunity but it brings up the question of the wisdom of using them as a guide in immunization with the colon-typhoid group.

When injected into the normal, vaccinated, or immune rabbit, the virulent hog-cholera bacillus is rapidly clumped and disappears from the circulation. 40 minutes after injection these organisms can be found in phagocytes in the liver. The fact that the normal rabbit gives this *intra vitam* agglutination is an exception to the findings of Bull that virulent organisms remain in the circulation for some time after injection.

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